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THE RELATION OF MYCORRHIZAE TO CONIFER SEEDLINGS[1]

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INTRODUCTION

Foresters are becoming more and more interested in the physiological relationships between forest trees and soil-inhabiting fungi. Certain soil fungi have long been known to be detrimental to tree seedlings as, for example, species of Pythium and Fusarium which cause damping off of succulent young seedlings. But the belief that all soil-inhabiting fungi are injurious to seedlings is no longer tenable. It is now believed that certain failures in forest nurseries and certain of the indifferent results sometimes encountered in planting out nursery stock are not due to the presence of a fungus but on the contrary should be attributed to the absence of some particular fungus.

Many pathologists believe that the association of certain fungi with the young root tips of trees and other plants to form mycorrhizae constitutes a definite case of parasitism on the part of the fungus. Other investigators are equally convinced that this association of two dissimilar organisms is distinctly beneficial to both participants. In any event, it is inconceivable that the formation of mycorrhizae should have no effect whatever on the health of the higher plants when all, or nearly all, of their rapidly growing root tips are invaded by fungous mycelium, with consequent swelling and forking of the roots. The occurrence of mycorrhizae on an astonishingly large number of plant species makes this question of the physiological rôle of mycorrhizae unusually important.

HISTORICAL DATA

The presence of fungus hyphae in the root cells of plants was described by numerous investigators during the first half of the nineteenth century. There were many speculations concerning the origin of these mycelial threads but no evidence of value until Reese (*26*)[2] in 1880 noted the apparent connection between a spruce rootlet and a truffle fungus, *Elaphomyces granulatus*.

In 1885 Frank (*3*) coined the word "mycorrhiza," and in a series of papers beginning in April of that year developed the hypothesis that a beneficial relationship might exist between the higher plant and the

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[2] Reference is made by number (italic) to Literature Cited, p. 315.

fungus. It is now generally considered that knowledge of mycorrhizae begins with the publication of Frank's researches. Frank, however, was not the first to advance the concept of a beneficial relationship between fungi and the roots of higher plants, for Pfeffer (*24*) in 1877 ascribed to the orchid fungi a physiological rôle analogous to that of root hairs, and Kamienski (*9*) in 1882 published a memoir on Monotropa in which he fully recognized the existence of a reciprocal relationship between flowering plants and fungi. These works have been overshadowed considerably by Frank's publications.

The interest in mycorrhizae that was aroused by Frank's papers has persisted and increased. More than 300 persons have written a total of approximately 600 papers dealing directly or indirectly with this subject.

It is now generally accepted that root infection of the mycorrhizal type is a widespread phenomenon among vascular plants. Mycorrhizae have been found among the Pteridophytes on ferns and on Equisetum; they have been found on the roots of orchids, violets, heaths, and a host of other small plants; also, on the roots of a great many species of forest trees, both hardwoods and conifers, and of fruit trees.

Several types of mycorrhizae have been pictured and described. The three types usually mentioned are ectotrophic, endotrophic, and ectendotrophic. The terms "ectotrophic" and "endotrophic" were first used by Frank; Melin (*17*, *18*, *19*, *21*) originated the term "ectendotrophic." Ectotrophic mycorrhizae are common on forest trees, expecially conifers. They are characterized by a fungous mantle around the root tip and the presence of mycelium between the cells of the root. In endotrophic mycorrhizae the hyphae are found inside the root cells and the hyphal strands pass through the cell walls from one cell to another; this type of mycorrhiza is found most often on herbaceous plants and hardwood trees. The characteristics of the endotrophic and ectotrophic mycorrhizae are combined in the ectendotrophic form. McDougall's (*14*) term "heterotrophic" is perhaps comparable to ectendotrophic. Rayner (*25*) believes that the type of mycorrhiza formed depends upon the degree of infection of the root by the fungus and intimates that a given fungus might form ectotrophic mycorrhizae on one plant and endotrophic mycorrhizae on another. Melin considers that the different types of mycorrhizae (ectotrophic, endotrophic, and ectendotrophic) represent phases in development. He believes that the ectotrophic condition is the final stage in a gradual "squeezing out" process due to enzyme activity in the root cells. The endotrophic mycorrhiza with its intracellular mycelium may, therefore, be transitional to the ectendotrophic type and this may be followed by the true ectotrophic type. Melin also states that the type finally developed probably depends on the "virulence" of the fungus. According to Melin, these intracellular filaments eventually disappear, and he assumes that they are digested and utilized by the root cells. Masui (*15*, *16*) describes this digestive process as consisting solely of a degeneration of the filaments due to the gradual granulation of the cell membrane of the hyphae until at last it refuses to take the stain and hence no longer can be seen.

The seasonal occurrence of mycorrhizae has been studied by both McDougall and Masui. These investigators found that mycorrhiza formation reaches a maximum in late summer or early fall and

thereafter steadily declines to a minimum during the winter months.

An imposing number of fungous species are reported as being mycorrhiza formers. Approximately 50 species representing 16 genera are in this list, and practically all are Basidiomycetes, chiefly agarics.[3] The genera mentioned most frequently are: Amanita, Boletus, Cortinarius, Cantherellus, Inocybe, Russula, and Tricholoma. Unfortunately, very few of those who have reported certain fungi as mycorrhiza formers have based their opinions on experimental evidence. The writer can suggest at least a dozen species not yet reported as mycorrhiza formers that careful field examination discloses to be closely associated with Norway spruce (*Picea excelsa*) and northern white pine (*Pinus strobus*). It is evident that many statements encountered in the literature must be accepted with reservation until verified by laboratory experiments.

Two methods have been used to determine whether a certain fungus is a mycorrhiza former. By one method strands of mycelium from a fungous fruit body are followed through the soil to a mycorrhiza; by the other mycorrhizae are formed in pure culture by inoculating the roots of seedlings with fungous mycelium.

The chief objection to the first method is the great difficulty in following the strands of mycelium through the soil without breaking the filaments. Moreover, there is no assurance whatever that the mycelium followed to the root is the mycelium which is responsible for the formation of the mycorrhiza. As will be brought out later, even the presence of a fruit body directly on a root and apparently in the most intimate contact with it does not necessarily signify that the fungus forms mycorrhizae.

In the second method the mycelium designed for inoculation purposes is obtained either from cultures of known fungi or from cultures originating with a mycorrhiza. Inoculations are made on the roots of seedlings that presumably are sterile, or on the roots of seedlings germinating from disinfected seeds sown within a flask or other closed vessel. A few of the investigators—Fuchs (*4*), for example—who obtained mycelium from the fruit bodies, did so by germination of the spores, but usually this mycelium has been obtained directly from the fruit body by culturing tissue taken from the interior of the fruit body. To obtain a pure culture of mycelium from a mycorrhiza is a difficult task and can not always be accomplished. Melin has developed a method for culturing mycorrhizae which he asserts is successful in sterilizing the external parts of the root and thus excluding those species (e. g., of Penicillium, Fusarium, and Mucor) which would be likely to outgrow the fungus causing the mycorrhiza. Fresh mycorrhizae are washed several times in sterile distilled water and then treated for 15 to 30 seconds with a 0.1 per cent solution of mercuric chloride, after which they are again washed in sterile distilled water and placed on the culture medium. Masui also has used this method with success. Möller (*22*) extracted mycelium from a spruce mycorrhiza and reinfected spruce rootlets with this mycelium. He inoculated with nonseptate mycelium which he thought might be a Mucor, but the mycelium which he later found inside the roots was septate. Möller's method of obtaining sterile rootlets for inoculation purposes

[3] According to Melin, certain incompletely identified species of Rhizoctonia may form "false" mycorrhizae. A few species of Ascomycetes have been reported as being mycorrhiza formers.

was to make a column of three pots of sterile sand and start the seedlings in the highest pot; when the roots had grown downward through two pots and had entered the third, they were inoculated by smearing mycelium on them.

Peklo (*23*) obtained a fungous culture from beech mycorrhizae by making a decoction of the mycorrhizae. Sections of beech roots which had been rinsed in water were dropped into this decoction and on these bits of roots he obtained a fungous mantle composed of several species of Penicillium. Peklo's results are of questionable value because he used seedlings which were grown for two years in unsterilized humus and may have been infected. Furthermore, Peklo and some of the other investigators failed to take into account the possibility that several species of fungi might be associated with the roots and none of them be able to form mycorrhizae. A few years later, in 1911, Fuchs found this to be true, and he declares that the fungous mantle sometimes developed by certain species of fungi is no sure evidence of mycorrhiza formation because the hyphae do not penetrate even the outer cells of the root, and the mantle is easily washed off. He cites Penicillium as an example of a fungus that forms a mantle which does not penetrate the root and hence is not mycorrhizal. When mycelium is extracted from a mycorrhiza there is no assurance that only one fungous species has been obtained, and unless the cultivated mycelium can be made to form a fruit body there is no way to know what fungus is being cultured. Finally, there is the possibility that the fungus really responsible for the formation of the mycorrhiza can not be extracted from the mycorrhiza; certain species (e. g., of Amanita and Cortinarius), which are strongly suspected of being mycorrhiza formers, grow very slowly or not at all in artificial culture.

Most of the recent investigators have begun their experiments with the seed, either raising seedlings under sterile conditions and transplanting these at the time of inoculation, or raising one or a few seedlings in a flask or test tube and inserting the inocula into these vessels. The latter method was used by Melin and Fuchs. Masui has used both methods, sometimes inoculating the seedlings when transplanted and at other times several months after transplanting.

Some investigators disinfected the seeds before planting, but others have not taken this precaution. Seed disinfection methods have varied, but solutions of mercuric chloride appear to meet with most favor. Masui tried several methods and settled on one involving the washing of green cones (containing germinable seeds) in 50 per cent alcohol, then in a 0.1 per cent solution of mercuric chloride. The seeds were removed while the cones were in the mercuric chloride solution and were then washed in sterile distilled water. Fuchs' method was much more elaborate. The seeds were shaken in 50 per cent alcohol, then in concentrated sulphuric acid for 5 to 10 minutes. They were then washed in a suspension of calcium carbonate to neutralize the acid, were rinsed in water, immersed for five minutes in 1.0 per cent mercuric chloride solution, and again rinsed in water.

In the first experiments with mycorrhizae, Frank used earthen flowerpots and Möller also used pots. Most of the latest experimentation have been done in Erlenmyer flasks. Melin has used a double flask made of one Erlenmyer flask and one Florence flask connected by a glass tube. The culture is grown in the Erlenmyer flask and a

reserve supply of nutrient solution is kept in the other flask. The object in using flasks is to make certain that the cultures remain absolutely sterile. Melin candidly admits, however, that in spite of all precautions at least 25 per cent of his flask cultures were contaminated by the end of the third growing season.

Nearly every experimenter has remarked upon the difficulty of obtaining a culture medium that is suitable for both seedling and fungus. Sand has been used chiefly because it is easy to sterilize and is inert. The latter characteristic is important when the effect of various nutrient substances on plant and fungus development is being tested. Although sand is suitable for the seedlings, some difficulty is usually experienced in getting the fungi to grow through it unless it is in very coarse particles. Humus and forest soils containtaining humus have been used, but these media are difficult to sterilize without seriously changing their chemical composition; often substances toxic to both seedlings and fungi are produced when humus is steam sterilized; chemical sterilization is considered impractical. Various attempts have been made to increase the porosity of sand used as a culture medium. Among the substances which have been mixed with it to make it more porous are glass beads and moss. Melin placed sand in flasks and sterilized these for 25 minutes in a steam sterilizer on each of three days. Fuchs also used sand in flasks and autoclaved these for 2 hours at 150° C.; he then mixed the treated sand with the nutrient solution and again sterilized the filled flasks for one-half hour. Masui used sand, and also a mixture of sand, humus, and sphagnum moss. These media were autoclaved in flasks for 30 minutes at 150° C.

So far as is known, Melin's 3-year experiments are the only ones lasting more than one year. The shortest experiments probably are some of those performed by Fuchs; these lasted but eight days. Masui's experiments were ended two or three months after the inoculations. Von Tubeuf (*31*) made experiments continuing for one year.

The effect of the association of a root and a fungus is one phase of this subject which has had a great deal of attention and about which there is yet very little definite information. Since Frank first raised the question as to the probable value of the fungus to the higher plant, our knowledge of this phase has gone very little beyond the theoretical stage. Nearly every conceivable hypothesis has been advanced as to the rôle of mycorrhizae in plant nutrition. The investigators who hold that the formation of mycorrhizae is injurious to the host plants include Sarauw (*29*), Möller, (*22*), Fuchs (*4*), Masui (*15, 16*), and McDougall (*14*). Frank (*3*), Stahl (*30*), Von Tubeuf (*31*), Rexhausen (*28*), and a number of others think the formation of mycorrhizae is beneficial or even necessary to the best development of the associated higher plant.

For about 30 years there was little compromise between these diametrically opposed ideas. But in 1909, Bernard (*2*) suggested that there might be a "balance of benefit" between the associated organisms, and that under certain conditions the association was beneficial to both participants, whereas under other conditions evidence of parasitism would appear. Since 1917 Melin has further developed the "balance of benefit" idea and has attempted to show that whether or not a mutual benefit is derived from the mycorrhizae

association depends very largely on the activity of the fungus. He points out that the various species of fungi which form mycorrhizae are not equal in their ability to form this union, but that some species are exceptionally vigorous while others are much less so. Furthermore, the external conditions which influence the health of the higher plant also have a part in determining in which direction the reacting organisms will proceed. Melin thinks that if a sickly plant effects an association with a particularly vigorous fungus, the plant may be injured and derive no benefit from the association; in a word, the fungus is parasitic upon the other plant. In short, the modern point of view is not that the formation of mycorrhizae must be either helpful or harmful to the participants of the association but that the effect of this association varies from absolute parasitism to complete symbiosis, depending upon the vitality of the fungus and the health of the higher plant.

The contradictory results obtained in investigations of the physiological significance of mycorrhizae probably are due to the methods used. In general, four methods have been employed to determine the effect on the plant of the fungus-root association. (1) Conclusions have been drawn from field observations. This admittedly is an inaccurate method, because allowance can not be made for all the many factors which simultaneously are influencing the development of the plant. (2) Conclusions have been based on the anatomical structure of mycorrhizae; but mycorrhizae are difficult to section and stain and even in a perfectly prepared slide it is easy to overlook important details. Many of the conclusions on the physiological rôle of mycorrhizae deduced from microscopic examinations should be verified by experiments with living plants. (3) Microchemical analyses have been made of infected and (supposedly) uninfected roots. Weyland (*33*) used these methods in studying the distribution of inorganic nutrient substances in root cells, and Masui has recently contributed an extensive paper on the subject (*16*). (4) There is the experimental method in which plant roots are brought into contact with the mycelium of a fungus suspected of forming mycorrhizae. By varying the conditions under which the plants are grown (as, for example, by supplying organic nitrogenous compounds to some plants and inorganic nitrogenous compounds to others), and by measurement of seedling height and needle length, observation of foliage color, and the like, direct evidence is obtained on the effect of mycorrhiza formation. In this field Melin is the outstanding investigator.

The investigators who have used the microchemical methods agree that the mycorrhizal fungi are parasitic upon the associated higher plants, and that there is no evidence of a symbiotic relationship. Masui, for example, found that the fungi remove from the root all of the amino acids, most of the carbohydrates, tannins, and nitrates, and some of the phosphorous, potassium, and ammonium. He found that young fruit bodies of the suspected fungi contained large amounts of these substances. Inasmuch as most of the important food substances were removed from the seedling by the fungus and no return of nutritive material was made, Masui was forced to conclude that the mycorrhizal fungus must be considered a parasite upon the seedling.

The evidence obtained by the experimental method is almost the exact opposite of that obtained by microchemical analyses. If the

experimental evidence does not always indicate a beneficial effect, it at least shows no parasitism on the part of the fungus. A full discussion of this phase of the subject is given in later paragraphs.

FIELD EXAMINATIONS

In order that laboratory experimentation might be tempered by a knowledge of mycorrhizal conditions as they obtain in nature, a systematic study was made in several forest plantations. These were located on the Saginaw Forest of the School of Forestry and Conservation, University of Michigan. After a preliminary examination in 1927 and 1928, when every conifer plantation was visited regularly several times each week over a period of about three months in the fall and again in the spring, it was decided to restrict the study to the plantations of Norway spruce and northern white pine.

Mycorrhizae were moderately plentiful in the white pine plantation and were truly abundant in the spruce plantation. The abundance of spruce rootlets in the top 6 inches of soil and the moist conditions prevailing in this stand undoubtedly provide a more favorable environment for the formation of mycorrhizae than the rather dry soil of the pine plantation. An estimate of the total amount of mycorrhizal infestation in each plantation was made periodically by digging up a number of small roots about 3 feet long in the top 6 inches of soil. At least 98 per cent of the rootlets on the better quality sites of the spruce plantation, and probably 75 per cent of the rootlets in the white pine plantation, were estimated to bear mycorrhizae. In neither stand were mycorrhizae found very far below the surface; only to a depth of perhaps 6 inches in the pine and to about 8 inches in the spruce. No mycorrhizae were found in that part of the spruce stand where raw humus is absent and the soil bakes hard and dry.

The pH values for soil and humus were determined in these two plantations in the fall of 1928, the spring of 1929, and again in the fall of 1929. Samples from different parts of the stands were tested promptly with the quinhydrone electrode. In Table 1 are given the average pH values for soil and humus in these two stands.

TABLE 1.—*Average pH values for soil and raw humus from the northern white pine and Norway spruce plantations on the Saginaw Forest*

Soil	Northern white pine		Norway spruce	
	pH value	Samples	pH value	Samples
		Number		*Number*
Raw humus	6.4±0.03	12	6.3±0.19	15
Soil at a depth of 4 inches	6.0± .03	18	5.3± .02	19

Hesselman (*7, 8*) and Melin (*20*) obtained pH values of about 4 in the humus layers of conifer forests in northern and central Europe and Gløimme (*5*) reports essentially the same values for conifer woods in Norway. According to Melin, Brenner found that soils in the conifer forests of Finland had pH values ranging from 3.5 to 4.8. In Sweden, Melin states, the best mycorrhizae and the most prolific occurrence of mycorrhizae are in forests where the raw humus has a pH value of

about 4. He also states that usually there is an abundance of mycorrhizae in spruce woods having pH values of about 5 for the soil, but that where the pH value is 6 or 7 the development of mycorrhizae is very weak. Melin's experiments lead him to believe that vigorous formation of mycorrhizae will not take place on neutral or on alkaline soils or in extremely acid soils (e. g., with a pH of about 3.5 or less). Lohman (*12*) has made a large number of collections of mycorrhizae from a wide variety of plants in Iowa and has determined the pH values for the soil surrounding each collection. These values ranged from 4.9 to 8.2 but were 6.5 or over in the case of more than 70 per cent of his collections. He concludes that mycorrhizae occur in acid, neutral, and alkaline soils but probably develop better and are more common in soils having pH values below 6.5.

The "glass plate" method of studying the seasonal development of mycorrhizae—first mentioned by McDougall—was tried in the spruce stand but was not entirely satisfactory for this purpose. Judging, however, from a large number of individual collections, the maximum development of mycorrhizae in these plantations occurs from early September to late November; during three successive years the best collections were had in October. The development of mycorrhizae apparently depends on abundant rainfall and warm weather; after a hot, dry summer a considerable fall of rain is needed to start a vigorous growth of the fungous mycelium. Excellent mycorrhizae were obtained two weeks before the appearance of fruit bodies in these areas. New mycorrhizae probably do not develop after the advent of cold weather and frozen soil, but those already formed may remain alive for several months; a number were dug from frozen soil in December, January, and February. By spring nearly all those formed during the previous year have turned brown, are more or less shriveled, and appear to be dead. Only a few mycorrhizae are formed during the spring and summer months.

In the Norway spruce stand were found seven 1-year-old spruce seedlings which apparently had originated naturally from seeds cast by the trees of this stand. No mycorrhizae were found on the roots of these seedlings.

In both the Norway spruce and northern white pine stands a great many attempts were made to verify the apparent connection between fungous fruit bodies and mycorrhizal roots. Many fruit bodies were found in actual contact with roots; others were directly over large mycorrhizal clusters and separated from the roots by only a centimeter or so. This apparently intimate association is no positive assurance that the mycelium which formed the fruit body also is responsible for the near-by mycorrhizae, but these associations do furnish a clue as to which fungi might well be tested experimentally to determine their mycorrhiza-forming ability. In the following list are given the names of species[4] which careful and repeated examination disclosed to be nearly always associated with tree roots in these two stands. The list also indicates the fungi suspected of forming mycorrhizae of the different types found in the microscopic examinations of specimens from the two plantations. These species represent only a part of the large number of fungous species collected in the two areas.

[4] The identification of these fungi, without regard to the mycorrhizal types they may form, has been checked by C. H. Kauffman.

Ectotrophic mycorrhizae:
- On Norway spruce—
  - *Amanita muscaria* Fr.
  - *Lycoperdon pulcherrimum* B. and C.
  - *Inocybe eutheloides* Pk.
  - *Clitocybe rivulosa* Fr. var. *angustifolia* Kauff.
  - *Clitocybe diatreta* Fr.
  - *Calvatia saccata* Vahl.
  - *Boletus piperatus* Bull.
- On white pine—
  - *Cortinarius* sp.[5]
  - *Calvatia saccata* Vahl.
  - *Lycoperdon gemmatum* Batsch.
  - *Collybia butryacea* Fr.
  - *Inocybe eutheloides* Pk.
  - *Clitocybe rivulosa* Fr. var. *angustifolia* Kauff.
  - *Clitocybe diatreta* Fr.

Ectendotrophic mycorrhizae:
- On Norway spruce—
  - *Cortinarius argentatus* Fr.
  - *Cortinarius cinnamomeus* Fr.
  - *Tricholoma personatum* Fr.
  - *Lepiota naucina* Fr. (or ectotrophic only?)
  - *Lycoperdon gemmatum* Batsch. (or ectotrophic only?)
- On white pine—
  - *Lycoperdon gemmatum* Batsch. (or ectotrophic only?)

Of these species, *Amanita muscaria* has been reported by Melin to form mycorrhizae on Betula, Larix, *Pinus sylvestris*, and *Picea excelsa; Cortinarius cinnamomeus* has been reported by Masui to form mycorrhizae on *Pinus densiflora* and *Populus tremula;* the rest, so far as is known, are mentioned for the first time in this connection.

It has been the common practice of investigators to consider the attachment of fruit bodies to tree roots as definite proof of the ability of these fungi to form mycorrhizae. It is realized that circumstantial evidence of attachment is often very strong, but the apparent attachment of fruit bodies to tree roots can not be taken as proof that the fungus forming the fruit body likewise formed the mycorrhizae. The writer has made a microscopic examination of 28 fruit bodies of *Lycoperdon gemmatum, Amanita muscaria, Calvatia saccata, Inocybe eutheloides, Lycoperdon pulcherrimum,* and *Cortinarius* sp., each with tree rootlets attached. In spite of careful sectioning, staining, and examination, in none of over 100 slides could the mycelium of the fruit body be followed to the interior of the root (*13*).

One hundred and eight individual collections of mycorrhizae from the white pine and Norway spruce plantations were embedded in paraffin, sectioned, and stained. There was sectioned also one collection from each of the following species: Austrian pine (*Pinus nigra* Arnold), Scotch pine (*P. sylvestris* L.), ponderosa pine (*P. ponderosa* Laws.), Douglas fir (*Pseudotsuga taxifolia* Britt.), and eastern hemlock (*Tsuga canadensis* (L.) Carr).

The mycorrhizae found in the spruce plantation were simple or coralloid. The simple form is doubtless an early stage of the coralloid form; the root is unbranched and the mantle is very thin. The coralloid form (the "Gabelmykorrhiza" of Melin) is characterized by a corallike branching of the rootlets and the presence of well-developed hyphal mantles.

[5] An undescribed species which Doctor Kauffman has had under observation for several years.

The mycorrhizae of spruce, which presumably are formed by *Amanita muscaria*, are markedly coralloid in form. At maturity they are invested with a mantle of pure white mycelium which turns brown with age and eventually sloughs off as the mycorrhizae die and begin to shrivel. The mycorrhizae are about medium size, from 3 to 5 mm long. Microtomic sections show the hyphal mantle to be comparatively thick, from 15μ to 30μ, and composed of a tangle of hyphal filaments which lie closely appressed to the rootlet. The mycelium penetrates between the cortical cells of the root as far, usually, as the central cylinder.

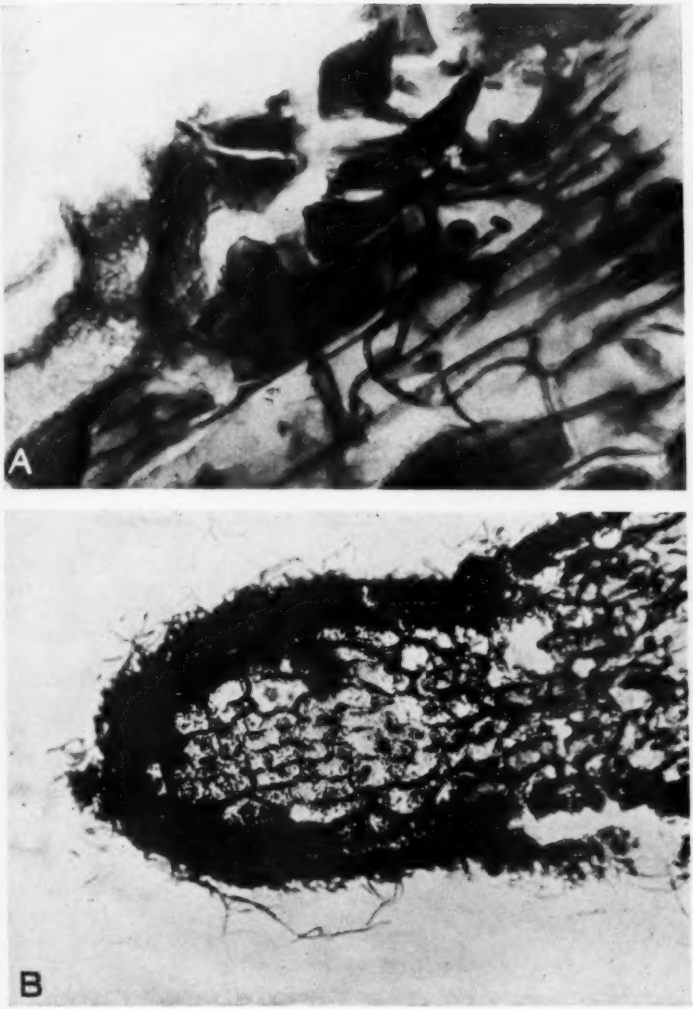
The mycorrhizae of Norway spruce, which probably are formed by *Cortinarius argentatus*, are coralloid and have a yellowish-white mantle. Numerous short hyphal projections give these mycorrhizae a hairy appearance. The mycelium of the mantle soon turns brown, then blackish, and finally disappears as the mycorrhizae die. Microscopically, these mycorrhizae are noteworthy because of the presence of mycelium within the cells as well as between the cells. (Pl. 1, A and B.) Until recently it was not believed that ectotrophic mycorrhizae might contain intracellular hyphae, but as Melin has pointed out, this frequently occurs; the proper staining is necessary to make the filaments visible. In this particular mycorrhiza, thought to be formed by *C. argentatus*, no evidence of digestion of the hyphae could be found.

The mycorrhizae of Norway spruce which *Lycoperdon gemmatum* is suspected of forming are coralloid, but less so than those apparently produced by *Amanita muscaria*. They are about 5 mm long and have a cream-colored mantle with numerous projecting hyphal filaments. The mycelium is intercellular, but numerous short, stubby, haustorialike branches of hyphae apparently penetrate the cell walls. It could not be determined whether actual penetration of the walls took place or whether the walls were distended inward without penetration. The hyphae were everywhere between the cell walls of the cortex but did not enter the central cylinder of the root.

Tricholoma personatum is suspected of forming pale-yellow mycorrhizae on Norway spruce. These mycorrhizae are distinctly coralloid and are characteristically bulbous at the tips. The swollen tip is sometimes twice the diameter of the rest of the mycorrhiza. The mycorrhizae are rather long, from 5 to 7 mm, and the mantle is smooth, from 10μ to 20μ in thickness, and composed of closely appressed filaments. The mycelium is both intracellular and intercellular, thus placing this mycorrhiza in the ectendotrophic class.

The mycorrhizae of spruce thought to be formed by *Clitocybe rivulosa* var. *angustifolia* are sparsely coralloid and were the least branched of all the mycorrhizae studied. When fresh, the mycelium of the mantle is grayish white and slightly fluffy; the mantle turns brown with age and appears to loosen and slip from the root as the mycorrhiza dies. The mantle is from 8μ to 15μ in thickness. These mycorrhizae are short, from 2 to 4 mm long, and the mycelium is exclusively intercellular, penetrating the root as far as the central cylinder.

Some of the mycorrhizae found in the northern white pine plantation were fully as coralloid as the *Amanita muscaria* (Norway spruce) mycorrhizae, but others found in this stand were almost tuberculate (the Knollenmykorrhiza of Melin). In no instance, however, were these tuberlike mycorrhizae joined together, as often occurs with the



Longitudinal sections through mycorrhizae on Norway spruce, suspected of being formed by *Cortinarious argentatus*, showing mycelium within cells of the root. A, magnified 700 times; B, 260 times

Knollenmykorrhiza type. Where the same (suspected) species of fungi were present as appeared in the spruce areas, the white pine mycorrhizae had much less conspicuous mantles, although microscopic examination showed that the mantles often were just as thick.

The mycorrhizae of white pine supposedly formed by *Collybia butryacea* were rather short (about 3 mm) and were invested with a grayish mycelial mantle from which numerous short filaments protruded. The mycelium penetrated between the cells of the cortex occasionally as far as the central cylinder; no intracellular hyphae were found. The mycelium inside the root was distinctly darker than the mycelium composing the mantle.

The mycorrhizae of white pine suspected of being formed by *Calvatia saccata* were tuberculate with occasional corralloid forms. These mycorrhizae were 4 or 5 mm long and had a thick mantle of cream-colored mycelium which appeared to darken with age; the mantle was not found on old (apparently dead) mycorrhizae. This mantle was from 12μ to 20μ in thickness and was composed of very closely interwoven filaments. The mycelium was exclusively intercellular and penetrated the root as far as the central cylinder. It was much darker inside the root than outside.

FORMATION OF MYCORRHIZAE UNDER CONTROLLED CONDITIONS

GROWING SEEDLINGS IN THE LABORATORY

Seedlings of Norway spruce and white pine were grown in pots of sand supplied with nutrient solutions. The seeds were first disinfected by covering them with a 0.25 per cent solution of mercuric chloride and shaking vigorously for a few moments to insure perfect contact of the solution with every seed. After 45 minutes the solution was poured off and the seeds were rinsed in three changes of sterile distilled water. The disinfected seeds were planted in quartz sand. The sand and the new earthen pots containing it had been sterilized by autoclaving for 3½ hours at 15 pounds pressure (121° C.). Northern white pine seeds, especially when fresh, were extremely erratic in germinating, but alternate wetting and drying greatly hastened their germination. For the spruce the germination averaged about 65 per cent and for the white pine about 50 per cent. The age of the seedlings has been computed from the time when germination began to show a marked decrease.

The nutrient solution used was that developed by Reid *(27)*.[6] When a solution without nitrogen was required, the nitrates of the Reid solution were omitted. For solutions having nitrogen from an organic source, the nitrates were replaced by asparagine, glycine, uric acid, or peptone. These solutions were applied to the seedlings every 10 days. The plants were watered with distilled water.

Various experiments were made to determine approximately the requirements of the seedlings for light. Direct sunlight was kept from the seedlings because of the considerable increase in temperature accompanying sunlight. Artificial light (supplied by 50 w electric lamps) was used to supplement the indirect sunlight reaching

[6] The chemical composition of the solution is as follows: Solution A—$MgSO_4$, 2 per cent; KH_2PO_4, 2 per cent; KNO_3, 2 per cent. Solution B—$CaCl_2$, 3 per cent; $CaSO_4$, 2 per cent; $Ca(NO_3)_2$, 4 per cent. Equal quantities of the solution are mixed and then diluted 20 times. A few drops of 1 per cent ferric citrate solution is added to the diluted solution.

the seedlings. It was found by experiment that continuous light—at least, over a period of one month—was not harmful to the seedlings; on the contrary, continuous illumination stimulated the development of new foliage.

Experiments were made to learn at what air temperature and relative humidity the best development of the seedlings takes place and at what point the seedlings begin to suffer from the influence of these two factors. It was found that rapid growth occurred with temperatures approximating 18° to 22° C. and a relative humidity above 35 per cent. The seedlings suffered when the temperature was above 24° and also when the relative humidity was below 20 per cent. The harmful effect of heat was offset by a high relative humidity, the maximum safe temperature being about 30° in a saturated atmosphere. It was observed also that a moist atmosphere encourages the development of new foliage and that dry air retards it. Because of this, the air temperature of the laboratory was kept as near 20° as possible, the lights were not used when the temperature unavoidably rose above 22°, and the air was kept moist by sprinkling water on the floor. Unless these precautions are taken it is likely that seedlings will die, and in some instances their death might erroneously be attributed to the action of a fungus if culture experiments are in progress.

In an experiment to determine the effect of the nitrogen source on the development of the seedlings, one set of seedlings was supplied with inorganic nitrogenous compounds, another set was furnished nitrogen in organic compounds (asparagine, peptone, glycine, and uric acid), and a third set was supplied with a nutrient solution containing no nitrogenous compounds. The seedlings with nitrates developed normally; at the end of the experiment (six months) they had comparatively short but many-branched roots, foliage of a dark-green color, and had increased about 1½ inches in height. The seedlings without nitrogen had a sickly appearance; their roots were long and sparsely branched, the foliage was yellow with many needles dropping, and they had grown scarcely half an inch in height. The seedlings supplied with nitrogen from organic compounds likewise exhibited signs of nitrogen starvation.

GROWING FUNGI IN PURE CULTURES

A number of fungi were cultured by placing fragments of clean tissue from the interior of fruit bodies on nutrient agar in Petri dishes. When sufficient mycelium had developed in these initial cultures, subcultures were made in other dishes and in test tubes. The best cultures were obtained from fresh, young fruit bodies collected during the period of maximum fruit-body production (in October); mycelium obtained from fruit bodies collected much earlier or later grew very slowly. The following fungi were cultured:[7]

Clitocybe rivulosa Fr. var *angustifolia* Kauff.
Clitocybe diatreta Fr.
Tricholoma personatum Fr.

[7] There was no reason to expect variations in the cultures of any fungus due solely to the location from which the fruit body was obtained. As a matter of record, the inoculation experiments to be described were made with cultures of fruit bodies obtained from the white pine and Norway spruce plantations, as follows: *Lycoperdon gemmatum* Batsch., from spruce stand, Nov. 8, 1928; *Tricholoma personatum* Fr., from spruce stand, Oct. 17, 1928; *Clitocybe diatreta* Fr., from pine stand, Nov. 4, 1928; *Calvatia saccata* Vahl., from spruce stand, Nov. 8, 1928; *Clitocybe rivulosa* Fr. var. *angustifolia* Kauff., from pine stand, Oct. 4, 1928.

Calvatia saccata Vahl.
Cortinarius sp.
Lycoperdon gemmatum Batsch.
Amanita muscaria Fr.
Boletus piperatus Bull.
Cortinarius cinnamomeus Fr.
Collybia butryacea Fr.

The initial cultures and the subcultures in test tubes were made on a nutrient agar.[8] Tests were made to determine the effect of various nutrient substances on the growth rate of the fungi.[9] Among the media used in these experiments were the following: Sugar agars which contained only matose, glucose, or sucrose and agar and water; an agar containing the compounds included in the Reid nutrient solution for seedlings; humus agars containing a decoction made by boiling raw humus from the spruce plantation; asparagine agar, in which the nitrogenous compounds of the regular nutrient agar were replaced by asparagine; Leonian agar;[10] malt extract agar; and several "tannin" agars made by adding to the media mentioned a little concentrated solution (of tannin and probably other substances) extracted from cork by boiling it in water.

Sucrose appeared to be less adapted to the needs of these fungi than either maltose or glucose, but no appreciable growth of mycelium was obtained unless a sugar was included in the culture medium. Most of the fungi made an extensive growth of mycelium on agar containing only sugar, but satisfactory development over a long period was not obtained unless mineral salts also were available. Although these fungi thrive in humus soil, none of them lived on the agar containing humus decoction; it is possible that boiling the humus may have produced substances toxic to the fungi. The addition of a little concentrated cork extract (probably consisting chiefly of tannin) to the culture medium materially aided the mycelial development of all fungi. The regular nutrient agar (see footnote 8) and this agar with cork extract produced the most rapid development of mycelium.

The individual fungi varied greatly in rate of mycelial growth. *Clitocybe rivulosa* made the most rapid spread of mycelium; this fungus grew rapidly from the time that cultures were started. Other species—e. g., *Calvatia saccata*—spread very slowly for several days and then grew vigorously. *Boletus piperatus*, *Amanita muscaria*, and *Cortinarius* sp. made no appreciable growth on any of these media.

The mycelium developed most rapidly at temperatures approximating 22° C. At 15° all fungi grew very slowly, and mycelial spread likewise decreased very markedly when the cultures were kept at 30°. At 36° there was almost no increase in spread of the mycelium.

These results are for fungi in artificial culture and may not represent the actual development of the fungi when growing in their native habitats. *Amanita muscaria*, for example, normally may be a slow-growing species, but it undoubtedly grows much faster in nature than

[8] This agar had the following composition: Agar,[c] 15 g; maltose, 5 g; peptone, 0.1 g; $MgSO_4$, 0.5 g; $Ca(NO_3)_2$, 0.5 g; KH_2PO_4, 0.25 g; distilled water, [c] 950 cm^3.

[9] The cultures were kept at a uniform temperature of 22° C. and the mean radial growth of the mycelium was measured every day. For about half of the cultures an additional measurement was made by tracing the daily spread of the mycelium with wax pencil on the bottom of the Petri dish; these impressions subsequently were transferred to sheets of paper and the area of each "ring" obtained with a planimeter; this indicated the daily spread of mycelium in square inches. The results thus obtained agreed very closely with those obtained by measuring the mean radial growth.

[10] Leonian agar has the following composition: Agar, 15 g; malt extract, 6.25 g; maltose, 6.25 g; $MgSO_4$, .62 g; KH_2PO_4, 1.25 g; distilled water, 1,000 cm^3.

[c] g and cm^3 are the abbreviations recently adopted by the Government Printing Office for grams and cubic centimeters, respectively.

in these cultures. A year after these experiments were made they were repeated with approximately the same results.

FORMATION OF MYCORRHIZAE IN PURE CULTURES

Several investigators have produced mycorrhizae in pure cultures, though not always in abundance. A résumé of the methods used has already been given. In the experiments now to be described a variety of media and several types of containers were used with the object of finding the culture medium and type of container best suited to long-time experiments of this character. The media employed were agar, sand, sawdust, cork, and combinations of these. Test tubes, flasks, and earthen flowerpots were used as containers for the fungus-seedling cultures.

Cultures in Test Tubes

Two types of cultures were made in test tubes. In one, disinfected seeds were placed on slanted nutrient agar and inocula added as the seeds germinated. In the other, a vigorous growth of mycelium first was obtained on slanted agar; then against this the roots of a sterile seedling were held in place either with sand or with sawdust.[11] When seeds were planted the tube was kept closed with a cotton stopper; when seedlings were used, the tube was filled to the top with the sand or sawdust and the top and most of the stem of the seedling protruded from the tube.

In these experiments with test tubes, mycorrhizae were formed on the roots of white pine and Norway spruce seedlings with *Tricholoma personatum*, *Clitocybe diatreta*, *C. rivulosa* var. *angustifolia*, and *Lycoperdon gemmatum*, none of which had been reported as a mycorrhiza former. The experiments gave no indication as to the physiological effect of mycorrhizae on the seedlings, for one set of seedlings (in sand) died while another set (in sawdust) lived when exposed to exactly the same species of fungi.

The experiments also revealed certain undesirable features in the use of test tubes. Raising seedlings from the seed within stoppered test tubes was not successful because aerial growth of the mycelium covered the foliage of the seedling. If sand is used as a restraining medium it must be used in larger quantity than can be contained in test tubes of the size employed (20 by 175 mm); unless this is done the sand will dry out and the seedlings will die. The indications are that sawdust is a better medium, physically, than sand for these experiments; it holds water better and, being more porous, permits a much better development of the mycelium than sand. In spite of these advantages, however, sawdust probably can not be used because of the difficulty of sterilizing it.

Cultures in Flasks

The first flask cultures were continuations of the attempt to form mycorrhizae in wholly inclosed cultures. Erlenmyer flasks of 500 to 1,000 cm^3 capacity were used. One of the following culture media was used in each flask: (1) A layer of sand about 2 inches deep, (2) a

[11] Subsequent mention of "sterile" seedlings implies that the seedlings were grown from disinfected seeds under sterile conditions in sterilized sand and before transplanting to cultures were washed quickly in 95 per cent alcohol and rinsed in sterile distilled water.

layer of granulated cork, (3) a layer of cork mixed with sand, or (4) a cork and sand mixture over a thin layer of agar bearing a vigorous growth of mycelium.[12] Disinfected seeds were planted in some of the flasks; sterile seedlings were transplanted to others.

The same species of fungi and seedlings were used as in the test-tube cultures to form mycorrhizae in these flasks. It was impossible, however, to continue the experiments for more than a few months because the humidity of the air within the flasks so promoted the aerial growth of the mycelium that the seedlings were smothered. Another difficulty was lack of drainage; seedlings and fungi died from excess moisture in the culture medium. A third difficulty was the rapid development of Penicillium and other contaminating organisms within the flasks—these pests did not appear in open cultures. Finally, it was impossible to maintain a large number of cultures.

An attempt was made to improve the moisture conditions within these flasks. For drainage, a short piece of glass tubing was fused into the side of the flask about half an inch above the bottom. To decrease the moisture content of the air within the flask an inverted U-shaped tube was inserted in the cotton stopper with one end of the tube inside the flask and the other end outside. These improvements overcame the excessively moist condition of the medium but did not materially reduce the atmospheric moisture within the flasks.

Simultaneous with these wholly inclosed cultures, another experiment was made using small (125 cm^3) Erlenmyer flasks and leaving the foliage and most of the stem of the seedling protruding from the flask. One hundred and four of these flasks were used, filled to the top with sand or with the cork and sand mixture. Half of the flasks were treated with a nutrient solution containing nitrogen in inorganic compounds (nitrates), and the others were given a solution containing nitrogen in an organic compound (asparagine). By leaving the foliage of the seedlings outside the flasks the aerial growth of mycelium was prevented. The experiment lasted two months and was discontinued because of the excessive mortality of the seedlings. Mycorrhizae were formed on the seedlings (pine and spruce) by the four species of fungi previously mentioned, but it was found by experiment that the seedlings died not because of the formation of mycorrhizae but because of the moist condition of the media in the flasks. In most of the flasks the medium was either very dry or was soggy with moisture. The living seedlings were in the few flasks containing media that were moderately moist. These results again demonstrated that drainage is a most important factor, and seedlings later were successfully grown in flasks having drainage holes in their bottoms.

Cultures in Pots

The experiments thus far performed demonstrated that certain fungi form mycorrhizae on northern white pine and Norway spruce.

[12] An effort was made to find some material which would make the sand more porous. Among the substances were glass beads, glass wool, a mixture of agar and sand stirred together while hot and allowed to cool, granulated cork, and a mixture of cork and sand. The mixture of cork and sand was found to be very satisfactory. The cork was boiled in small quantities for 10 minutes in each of three changes of water to remove most of the tannin and other soluble substances. A test showed that 21 changes of water did not entirely remove the tannin but 3 changes of water removed a very large part of it. As the experiments with fungous cultures demonstrated, cork extract, probably consisting mainly of tannin, does not inhibit the development of the fungi used in these experiments; on the contrary, it promotes the growth of the mycelium. After boiling, the cork was placed in flasks and autoclaved for one hour, then rinsed in two changes of sterile distilled water and dried at 105° C. for about four hours, or until dry. The mixture consisted of 1 part sand to 4 parts of granulated cork, by volume.

The results gave several equally important clues as to the requirements for a method which could be used in extended experiments. Not only must this method be adapted to cultures lasting two or three years, but the method must be so devised that, the physical conditions existing in the cultures being kept as constant as possible, any observed change in the condition of the seedlings could be attributed to some other cause. A mixture of cork and sand had proved to be a suitable porous culture medium; drainage was found to be absolutely essential to avoid impossible growth conditions for the seedling; also, aerial growth of mycelium could apparently be prevented by permitting the foliage of the seedlings to grow in the open.

The use of earthen flowerpots as containers for the cultures was suggested very early by the results obtained in the experiments with test tubes and flasks. Preliminary tests with flowerpots were started as soon as difficulties were encountered in the use of flasks and the necessary foundation in experience was thus laid in time to take full advantage of the conclusions finally drawn from the flask experiments.

Sixty 5-inch pots were prepared in a uniform manner. A shallow, V-shaped groove (for drainage) was cut in a new cork along the entire length of one side and the cork inserted in the large drainage hole of a new clay flowerpot. The pot was autoclaved for 3½ hours at 15 pounds pressure. Sand was autoclaved in flasks for 3½ hours and then heated at 105° C. for about 6 hours, or until dry. New granulated cork was boiled in three changes of water, autoclaved, rinsed, and dried in the manner already described. Subsequent operations were performed as rapidly as possible and under sterile conditions. The sand and cork were mixed in the pot in the proportion of 1 part of sand to 4 parts of cork by volume. Sterile nutrient solution was added until the cork and sand mixture was thoroughly moistened. Mycelium from cultures made on agar containing only maltose was stirred into the cork and sand mixture. Seedlings raised under sterile conditions from disinfected seeds were washed in 95 per cent alcohol, rinsed in sterile distilled water, and transplanted to the pots. A layer of sterilized dry sand was poured over the top of the cork and sand mixture in each pot and moistened with distilled water.

Seven seedlings were placed in each pot: 3 spruce seedlings 3 weeks old, 3 spruce seedlings 5 months old, and 1 seedling of white pine 6 months old. The fungi used were *Tricholoma personatum*, *Clitocybe rivulosa*, and *Lycoperdon gemmatum*.

Nutrient solutions were applied in three forms: (1) With inorganic nitrogenous compounds (nitrates), (2) with organic nitrogenous compounds (asparagine, uric acid, peptone, or glycine), and (3) without nitrogenous compounds. Each nutrient solution contained 0.5 per cent maltose.

For the series with inorganic nitrogenous compounds there were 4 pots for each fungus species and a check of 4 pots without fungi, a total of 16 pots and 112 seedlings. For the asparagine series there were 4 pots for each fungus and a check of 4 pots without fungi, a total of 16 pots and 112 seedlings. For each of the uric acid, glycine, and peptone groups there were 2 pots for each fungus and a check of 2 pots without fungi, a total of 8 pots and 56 seedlings for each of the three groups. For the nitrogen-free series there was 1 pot for each fungus and a check of 1 pot, a total of 4 pots and 28 seedlings. In all, 420 seedlings were used.

Another experiment was started in which single seedlings were planted in 2½-inch and 3-inch pots instead of 5-inch pots. In this series only spruce seedlings and *Tricholoma personatum* were used. It was decided as a result of this experiment that the smaller pots for single plants are best, since, if a seedling dies during the course of the experiment, it may then be removed for examination without disturbing other seedlings. The results of this experiment are included with those obtained with the larger pots.

Artificial light was supplied to the seedlings for about eight hours each day. Distilled water was sprinkled over the pots daily in sufficient quantity to keep the sand layer moist. The small groove in the cork stopper allowed surplus water to drain away gradually but prevented water and nutrients from passing too rapidly through the pot. Nutrients were applied every 10 days by making a small hole in the sand layer and pouring the solution directly into the substratum through a funnel. In previous experiments it had been found that any of the nutrient solutions used in this experiment could be poured over pots of sand without danger of having such a common laboratory pest as Penicillium appear on the surface of the sand. These contaminations did appear, however, on the sides of the pots just above the sand layer where nutrient solutions had fallen in sprinkling them over the pots. By applying the solutions directly to the substratum the tops of the pots were kept clean throughout the course of the experiment.

The intrusion of Penicillium of other contaminating organisms was guarded against as much as possible, but there was no assurance that they would not reach the interior of these cultures, nor any reason to believe that these organisms would not grow in the cork and sand layer. At close of the experiment, however, not one instance of such contamination was found in these pots. Furthermore there is no known instance of Penicillium or like pests forming mycorrhizae. Fuchs made a number of attempts to form mycorrhizae with several species of Penicillium which he had extracted from roots bearing mycorrhizae but was unsuccessful. He reports that the mycelium of these fungi did not penetrate the roots and even if a hyphal mantle was formed it could be washed from the root.

To check Fuchs' results, 7 pots of seedlings were prepared as for the regular experimental work and were inoculated with cultures of fungi as follows: (1) 2 pots with *Penicillium* sp. from an agar plate exposed in the laboratory, (2) 1 pot with *Penicillium* sp. cultured from undisinfected Norway spruce seeds, (3) 1 pot with *Rhizopus* sp. cultured from undisinfected Norway spruce seeds, (4) 1 pot with *Rhizopus* sp. from an agar plate exposed in the laboratory, and (5) 2 pots with the only Mucors available, *M. lamprospora* and *M. spinescens*. The pots were examined four months later and in no instance was there any evidence of mycorrhiza formation. Except for one pine seedling, the plants were all living in a healthy, vigorous condition. Microscopic examination of the roots of these seedlings, including the dead pine, disclosed no mycelium within the roots, and on only three seedlings was there an appreciable amount of mycelium on the exterior of the roots. As will be described shortly, mycorrhizae were formed in every pot inoculated with Basidiomycete fungi. It appears, therefore, that the ordinary contaminations likely to reach

cultures in this particular laboratory may be disregarded in so far as mycorrhiza formation is concerned.

The experiment with the large pots lasted four and one-half months. Mycorrhizae were formed on the roots of both pine and spruce seedlings in every inoculated pot. A few seedlings had less than 50 per cent of their rootlets infected, but on most of the seedlings all, or practically all, of the rootlets were infected with the fungous mycelium. The experiment again demonstrated that *Tricholoma personatum*, *Lycoperdon gemmatum*, and *Clitocybe rivulosa* var. *angustifolia* form mycorrhizae on white pine and Norway spruce seedlings.

The experiment gave no indication that the nitrogen source (i. e., whether from organic or from inorganic compounds) has any marked influence on the rapidity of mycorrhiza formation or the abundance of mycorrhizae. Mycorrhizae were formed just as rapidly and in as large numbers in the cultures with peptone as a nitrogen source as in the cultures with nitrates. In the cultures where nitrogen was omitted from the nutrient solution, mycorrhizae were formed with no less rapidity but in somewhat less abundance than in the cultures with nitrogen.

In the series in which no nitrogen (except, perhaps, from the cork) was given to the cultures, the seedlings developed normally for nearly two months and then in both the inoculated and check pots the foliage began to fade and there was a slight decrease in the development of the needles. Two pine seedlings of the inoculated pots and one pine and one spruce seedling of the check pots were dead at the end of the experiment. Irrespective of the presence of mycorrhizae, these seedlings without nitrogen had a sickly appearance.

In the nitrate series the seedlings in both check and inoculated pots were in excellent condition at the end of the experiment. Their foliage was a fresh, green color and new needles were developing rapidly. Absolutely no difference could be seen between the infected seedlings and the uninfected seedlings. One pine seedling died at the age of six weeks in a pot inoculated with *Lycoperdon gemmatum*. The other seedlings, including those in the check pots, were alive at the end of the experiment.

In the asparagine, uric acid, and glycine series the results were almost the same as with the nitrate series. No difference could be detected between the appearance of the infected and the uninfected seedlings. The foliage of these seedlings was dark green and the needle development was vigorous. One pine seedling with asparagine and *Clitocybe rivulosa*, and one spruce seedling with uric acid and *Tricholoma personatum* were dead one month after the start of the experiment.

In the peptone series the results obtained were similar to those of the nitrogen-free series. The foliage was yellowish green and the needles appeared to be somewhat shorter than in the other series. Except for the color of the foliage, these differences were not pronounced. As nearly as could be determined, there was no appreciable difference in the condition of the infected and the uninfected seedlings. One pine of the check set and one young spruce with *Tricholoma personatum* were dead one month after the start of the experiment.

The excellent seedling survival in these experiments with pots is noteworthy when compared with the results obtained in the experiments with flasks and test tubes. The survival with pots was over 98 per cent, and this includes the nitrogen-free cultures. Only two seedlings died in check pots, and these were in the peptone and the nitrogen-free series.

These experiments yielded no absolute proof that the presence of mycorrhizae is either detrimental or beneficial to the seedlings. Nor did the results support or refute the hypothesis that mycorrhizae aid seedlings to obtain nitrogen from complex organic compounds. The experiments did substantiate previous investigations which indicated that seedlings suffer from lack of nitrogen when this element is present only in complex organic compounds such as peptone.

DEVELOPMENT OF THE MYCORRHIZAL MANTLE

The development of the fungous mantle on seedling roots was studied in cultures made in glass-sided boxes. The most successful type of box was made of galvanized sheet iron and was 10½ inches tall, 8 inches wide, and 2 inches thick, with a sheet of plate glass in one of the larger faces. The glass surface was covered with black paper, except when an examination was being made. Difficulties in proper placement of seedlings were overcome as follows: A box was filled with sand and moistened thoroughly with nutrient solution; the box was then placed so that the glass side was uppermost and the glass was withdrawn from the box; the seedlings and inocula were placed as desired on the surface of the sand, and the glass plate carefully pushed back into the box. This box would be greatly improved if the sides were so hinged that the glass plate could be lifted directly from the sand and replaced without sliding.

In the tests made in these boxes mycelium on the roots grew very slowly unless in contact with the actively growing part of the root tip, when a pronounced increase in development promptly occurred. In most of the mycorrhizae the mycelium apparently reached the root at the tip, uniformly enveloped the tip, and then spread back over the root in a thin mantle at the rate of 2 or 3 mm a week. How rapidly the mantle increased in thickness is not known, nor has sufficient study been made to show how soon the mycelium begins to penetrate between the outermost cells of the root. In two months mycorrhizae were produced that were comparable to those of the regular pot cultures.

The location of the infected root tips was marked with wax pencil on the glass side, and in this way it was determined that the infected roots made no appreciable increase in length after the formation of the fungous mantle. The initial presence of the mycelium did not entirely stop the elongation of the root, but its rate of elongation suddenly decreased upon the formation of a rudimentary hyphal mantle.

Microscopic examination of older roots to which strands of hyphae apparently were attached disclosed no penetration of mycelium into the root cells. This indicates that mycorrhiza formation depends upon the presence of an actively growing root tip. Masui asserts that he found mycelium within older roots, but Melin was unable to verify this finding.

The mycorrhizae produced in pure cultures were killed and fixed in weak chromo-acetic acid solution; after washing, dehydrating, and embedding in paraffin, these mycorrhizae were sectioned on a

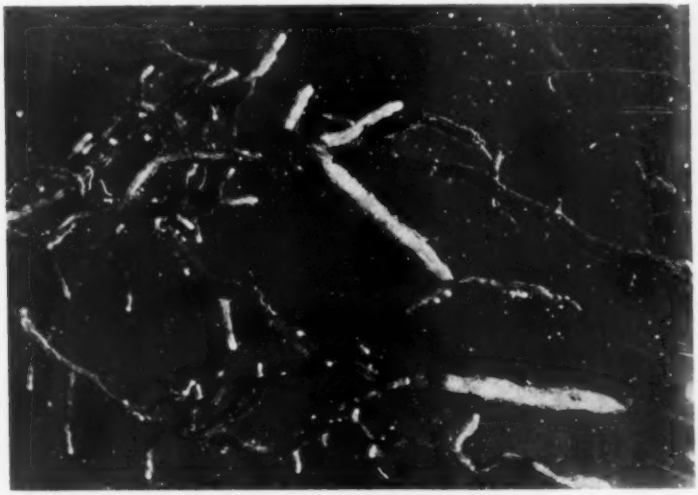


FIGURE 1.—Mycorrhizae on Norway spruce, formed in synthesis with *Clitocybe rivulosa* (asparagine series)

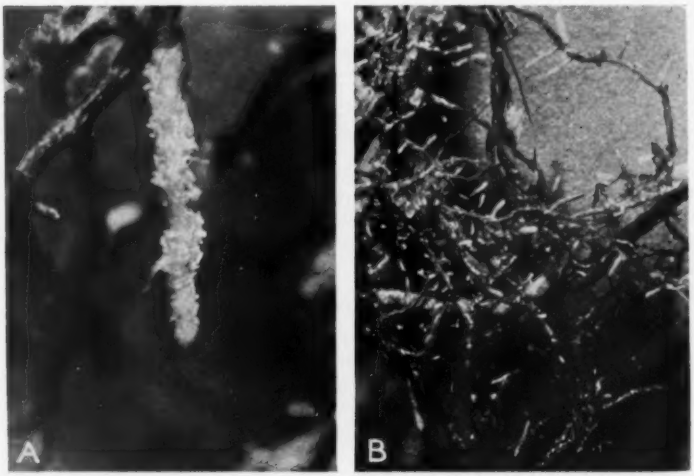
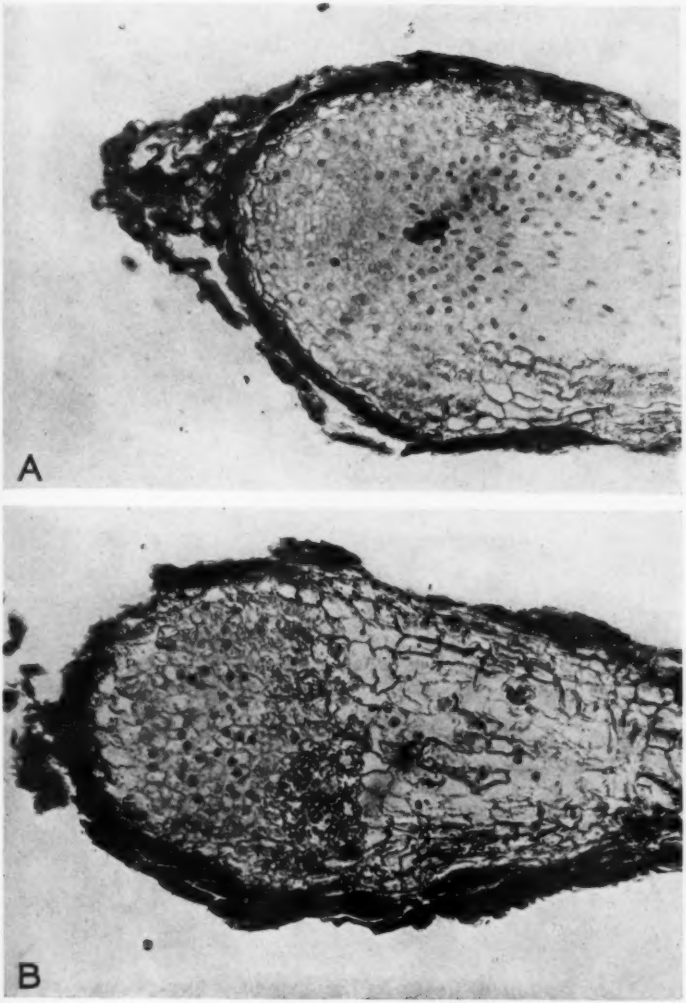


FIGURE 2.—A, Mycorrhiza on Norway spruce, formed in synthesis with *Clitocybe rivulosa* (asparagine series); B, mycorrhizae on Norway spruce, formed in synthesis with *Clitocybe rivulosa* (nitrate series)

microtome and stained in Delafield's haematoxylon and safranine or with Pianeze III (*32*).[13]

[13] The stain has the following composition: Malachite green, 0.5 g; acid fuchsin, 0.1 g; napthol yellow, 0.01 g; water, 150 cm³; and alcohol (95 per cent), 50 cm³.



A and B, Longitudinal sections through mycorrhizae formed on Norway spruce in syntheses with *Tricholoma personatum* (peptone series)

The mycorrhizae formed in the culture experiments closely resembled those collected in the field, except that the coralloid habit was less pronounced and the fungous mantles were somewhat thinner. The results so far obtained do not indicate any appreciable difference in the external appearance of the mycorrhizae which could be attributed to the nitrogen source.

The mycorrhizae formed on Norway spruce by *Clitocybe rivulosa* var. *angustifolia* were slightly coralloid, from 2 to 7 mm long, and had grayish white mantles with numerous short projecting strands of hyphae. (Figs. 1 and 2, A and B.) The mantle was only 4μ to 9μ in thickness and was composed of loosely interwoven filaments about 3μ in diameter, frequently septate, and with numerous clamp connections. Within the root the mycelium was exclusively intercellular and penetrated the root in some mycorrhizae as far as the

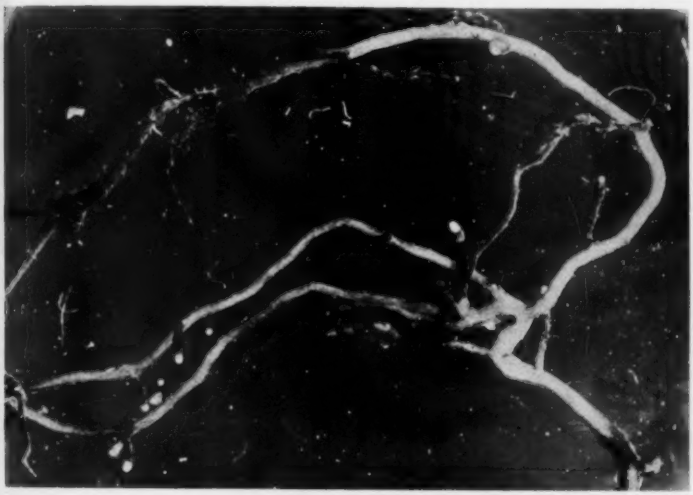


FIGURE 3.—Mycorrhizae on Norway spruce, formed in synthesis with *Tricholoma personatum* (nitrate series)

central cylinder; this intercellular mycelium was darker than that of the mantle.

The mycorrhizae of Norway spruce and *Tricholoma personatum* (figs. 3 and 4, and pl. 2, A and B) were much more coralloid than those produced on spruce by *Clitocybe rivulosa* (figs. 1 and 2). The mantles were yellowish white, with smooth outer surfaces, and were about 10μ or 12μ in thickness. The tightly interlaced hyphae of the mantles were about 3μ in diameter, had numerous clamp connections, and were frequently septate. Characteristic of these mycorrhizae were their distinctly bulbous tips; those collected in the field and suspected of being formed by this fungus also had bulbous tips. Except at the very tips of the roots the mycelium penetrated the root as far as the central cylinder and sometimes invaded that portion of the root. In practically all of the mycorrhizae examined, the mycelium was both intercellular and intracellular and was slightly darker inside

the root than in the mantle. No trace of the "digestion" of the intracellular hyphae could be found. The root cells in the cortical tissue contained minute granular bodies similar to those described by Masui, who expressed the opinion that these granules are com-

FIGURE 4.—Mycorrhizae on Norway spruce, formed in synthesis with *Tricholoma personatum* (asparagine series)

posed of a tannic substance. Melin describes essentially the same condition in mycorrhizae of *Pinus sylvestris*, but thinks that the granular bodies are secreted by the fungus. Masui found them, however, in normal, uninfected roots.

The mycorrhizae of Norway spruce and *Lycoperdon gemmatum* (fig. 5) were simple, but coralloid mycorrhizae occasionally were found. Their mantles were white, often with a pale-yellow tint, and the many short hyphal strands projecting from the mantles gave these mycorrhizae a hairy appearance. The mantles were approximately 6 mm long and from 10μ to 16μ thick. The mycelium was 3.5μ or 4μ in diameter, frequently septate but having only a few clamp connections and penetrating the root intercellularly almost to the central cylinder. In several instances an intracellular development of the mycelium was suspected, but this could not be established definitely.

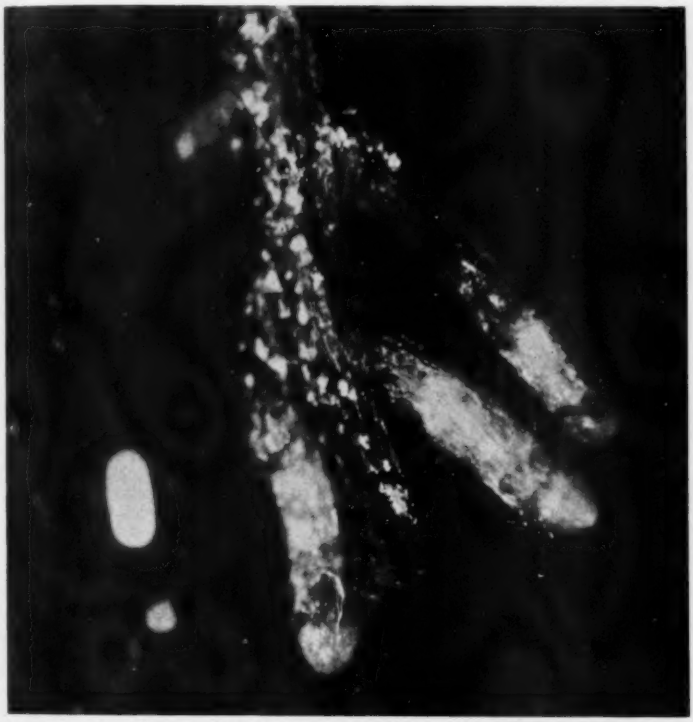


FIGURE 5.—Mycorrhizae on Norway spruce, formed in synthesis with *Lycoperdon gemmatum* (nitrate series)

The mycorrhizae of Norway spruce and *Clitocybe diatreta* were very similar to those formed on spruce with *C. rivulosa*, although the mantles were whiter. As with most of the mycorrhizae formed in culture the roots were but slightly coralloid. The mantles were composed of loosely interwoven hyphae about 3μ or 3.5μ in diameter, frequently septate, and with a great many clamp connections; numerous short filaments of hyphae projecting from the mantle

gave these mycorrhizae a hairy appearance. The root was penetrated intercellularly by mycelium as far as the central cylinder, and in a few instances mycelium was found in the central region of the root.

With one exception, the mycorrhizae formed on northern white pine by these four species of fungi were very similar to the corresponding mycorrhizae on Norway spruce, and require no separate description. (Fig. 6, A and B.) *Tricholoma personatum*, however, formed mycorrhizae with hairy mantles rather than the smooth mantles characteristic of the mycorrhizae which it produced on spruce. The bulbous tip of the spruce mycorrhizae also was less evident in the pine mycorrhizae formed by *T. personatum*. On white pine the mycorrhizal mantles developed by all of these fungi were thinner and less compact than those formed on spruce; the

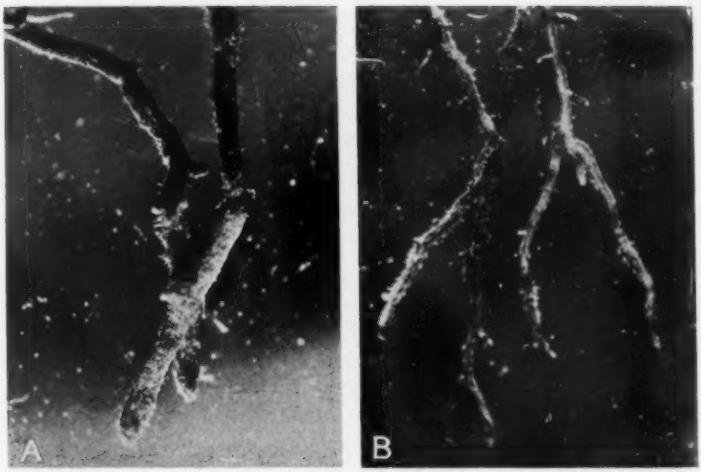


FIGURE 6.—A, Early stages of mycorrhiza formation on white pine, in synthesis with *Lycoperdon gemmatum* (nitrate series); B, mycorrhizae on white pine, formed in synthesis with *Tricholoma personatum* (nitrate series)

roots, however, were penetrated by the mycelium just as far in the pine as in the spruce.

DISCUSSION

Despite the numerous investigations which have been made there is very little known about the exact way in which mycorrhizae aid or harm the associated higher plants. Experimental evidence of unquestionable character is exceedingly sparse. Much evidence which might be acceptable because of accurate and careful methods must be taken with reservation because of insufficient data; conclusions of considerable importance have been based on experimentation with a very small number of seedlings, and in a few instances pages have been written on the results obtained with only one seedling. Especially when conclusions concerning the physiological significance of mycorrhizae are based on measurement of seedling

height growth, size of leaf, or similar characters which usually vary widely, a large number of seedlings must be used in order to escape errors due to random sampling and to offset the inherent differences present in certain species of trees. Furthermore, there must be as much infection on the roots of these seedlings as occurs in nature; the formation of mycorrhizae on 5 or 10 per cent of the rootlets of a seedling does not simulate natural conditions. Moreover, when seedlings die it must be known without doubt whether death was caused by the formation of mycorrhizae or other factors.

Although there is but little definite proof, there are excellent indications as to the influence of mycorrhizae on seedling development. There are indications, for example, that the fungus prepares complex nitrogenous compounds for absorption by the plant. In an analysis of seedlings having peptone (an organic nitrogenous compound) as a source of nitrogen, Melin found that seedlings with mycorrhizae contained 8 per cent more nitrogen at the end of three years than uninfected seedlings in similar cultures. Just how this transfer of nitrogen is effected has not yet been determined. According to Hesselman (*7*), the detritus in a conifer forest (that is, in the "raw humus" type) decomposes very slowly, with the liberation of ammonia from the organic compounds. Very little is known of the chemical composition of humus, and it is difficult to discuss a reaction of this sort without accurate knowledge of the organic compounds present in humus. At all events, it is generally conceded that very little ammonia is produced in raw humus and that most of the ammonia which is produced is captured by the various microorganisms present. Melin states that in raw humus soils the nitrogen content is low in proportion to the carbon and for this reason the microorganisms can compete with the higher plants for nitrogen. He considers that mycorrhizae act as absorbing organs for the tree and thus enable the tree to obtain nitrogen in spite of the competition by the microorganisms. Frank (*3*), Hesselman (*8*), and Von Tubeuf (*31*) also assert that mycorrhizae aid the tree in absorbing organic nitrogen.

The experiments described in this paper suggest that seedlings without mycorrhizae have difficulty in obtaining nitrogen when it is present only in some complex organic compound. These experiments, however, do not prove that the presence of mycorrhizae enables seedlings to obtain nitrogen from such compounds. Cultures lasting for two or three years may modify this statement.

Melin, Stahl, and Rexhausen reported that inorganic compounds were absorbed equally well by infected and uninfected roots. Stahl and several other investigators have emphasized the importance of the fungous sheath in facilitating the passage of water into the root. As a matter of fact, Stahl considered that the formation of mycorrhizae represented an ecological adaptation to soil conditions.

Melin has shown that neither seedlings without mycorrhizae nor those with mycorrhizae can assimilate free nitrogen from the atmosphere. These results are at variance with those of P. E. Müller, who held that his own experiments with *Pinus montana* indicated the ability of the tree to assimilate nitrogen from the air if the tree had mycorrhizae on its roots.

The fungi certainly benefit by association with the roots of seedlings. Hansteen-Cranner (*6*) states that the roots of higher plants excrete various substances, and other investigators have found that

the growth of fungi is accelerated when the fungi are in contact with the roots of plants presumably possessing such excretions. According to Barthel (*1*), Wilson found that the development of many species of bacteria is stimulated when they are supplied with root excretions. Melin discovered that the dry weight of fungous mycelium was from 40 to 56 times greater when grown in contact with seeds or seedlings than when grown alone. Another possibility, hitherto unconsidered and as yet unproved, is that the fungi utilize the large amount of organic material which is liberated from the root caps of plants by abrasion. It is not necessary for the fungus to penetrate the root to secure this material. After entering the root, the fungus undoubtedly obtains carbohydrates, especially glucose, and many other substances from the higher plant. Masui found that fungi obtain amino acids, carbohydrates, nitrates, phosphorus, potassium, ammonia, and tannin from the roots. Peklo also thought that fungi might obtain tannin from the roots, whereas Melin declares that the mycorrhizal fungi do not obtain tannin from the roots and are unable to use tannin. The experiments described in this paper indicate that the development of certain mycorrhizal fungi is accelerated by adding to their nutrient media tannic substances extracted from cork.

Irrespective of the precise manner in which the fungi or seedlings individually are aided or harmed by their mutual association, the general effect of the mycorrhizal association is of great importance. Particularly is this true with seedlings in forest nurseries. Kessell (*11*) reports the failure of a number of nurseries recently started in Australia on new soil. The seedlings in these nurseries germinated but soon turned yellow and were stunted in growth after reaching a height of about 3 inches. Experiments made to determine the cause of these failures failed to demonstrate that season of sowing, watering, shading, commercial fertilizers, pathological conditions, or hydrogen-ion concentration of the soil was responsible for the poor health of these seedlings. At this point a quantity of soil from an old nursery was dressed into the topsoil of the new nursery. After this the seedlings developed normally. The same results were obtained by planting seedlings from an old nursery among the seed beds on the new area. Kessell believes that the absence of the proper fungi to form mycorrhizae is the factor chiefly responsible for the failure of the sowings in the newly established nurseries. Kelley (*10*) reports a strikingly similar condition in a nursery established on new soil in Pennsylvania. The widespread occurrence of mycorrhizae on the roots of nursery seedlings has been noted generally. In three nurseries the present writer has found abundant mycorrhizae on the roots of practically all seedlings examined. (Fig. 7.) In view of the abundance of mycorrhizae it may be pertinent to inquire if a "normal" seedling can possibly be one without mycorrhizae?

It is the writer's opinion that the presence of mycorrhizae is beneficial to seedlings and probably also to trees in most instances. Although only a part of the total root system of a large tree is in the upper layer of soil where mycorrhizae are found, most of the small roots are in this upper stratum, and in this horizon are also many of the nutrient substances essential to plant life. Many species of trees undoubtedly rely on the roots inhabiting this shallow layer to supply

them with most of their mineral food substances. If mycorrhizae do not aid the tree in securing these substances, they at least do not appear to hinder the tree in obtaining them unaided.

As evidence of parasitism one may cite the cessation of growth in length of the infected roots and their apparent death after a few months. But if this argument is advanced, one must also be prepared to explain how it is that mycorrhizae can be so prevalent and

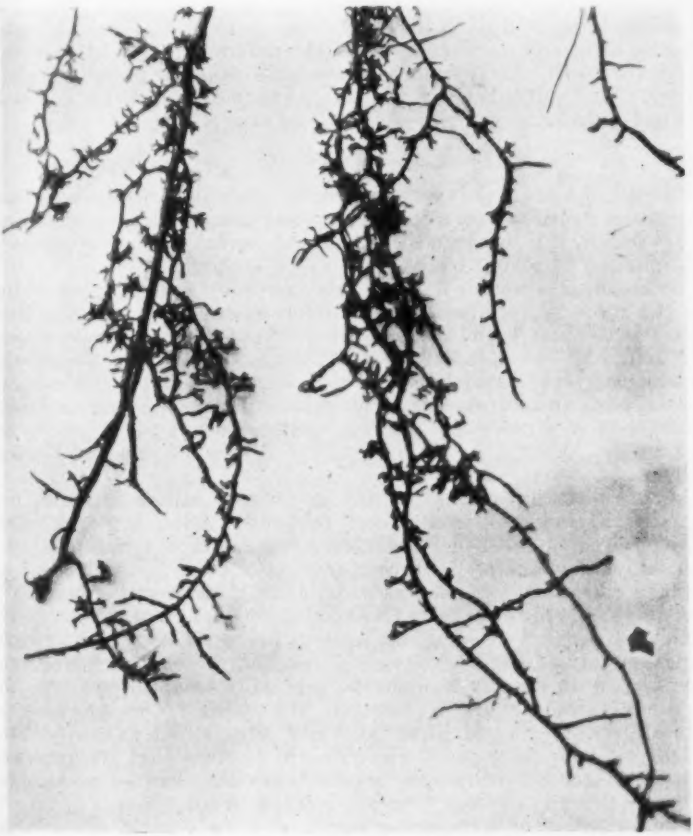


FIGURE 7.—Mycorrhizae on roots of 4-year-old white pine seedling from a forest nursery

yet not visibly injure the plants. No experimental evidence so far advanced proves beyond doubt that the presence of mycorrhizae is detrimental to trees or seedlings. On the contrary there is considerable experimental and circumstantial evidence to show that the formation of mycorrhizae either is not harmful to the trees, is beneficial to the trees, or is even necessary to the vigorous growth of the plants. The "balance of benefit" theory may not always hold, for

it seems illogical to attribute to a fungus ordinarily harmless the death of a seedling or tree that is dying for want of the proper food substances. The death of sickly seedlings has been reported as being due chiefly to fungous activity, but it was not stated that mycorrhizae actually were formed, and unless mycorrhizae were formed it is difficult to see how the presence of mycorrhizae could result in the death of the seedlings.

Finally the writer wishes again to emphasize the importance of basing conclusions on samples sufficiently large. A large number of seedlings must be used in these syntheses in order to eliminate experimental error and to offset the inherent differences present in certain species of trees. Furthermore, mycorrhizae must be present in abundance. The writer believes that large samples can be used if pots, instead of flasks, are employed for the cultures.

SUMMARY

Results of a field study of mycorrhizae made in several plantations of conifers include the seasonal occurrence of mycorrhizae, the various types found, the pH values for soil and humus, and a microscopic examination of a very large number of mycorrhizae.

By means of a technic devised for successfully forming mycorrhizae on the roots of seedlings grown under controlled conditions, such formations have been brought about repeatedly on the roots of northern white pine and Norway spruce seedlings in syntheses with *Tricholoma personatum*, *Lycoperdon gemmatum*, *Clitocybe rivulosa* var. *angustifolia*, and *C. diatreta*, and these fungi have been definitely established as mycorrhiza formers. Further, eight other species are suspected of forming mycorrhizae on northern white pine and Norway spruce.

Mycorrhizae have been formed in cultures where nitrogen was supplied by inorganic compounds (nitrates) and in cultures where nitrogen was supplied in organic compounds (asparagine, uric acid, glycine, and peptone). Mycorrhizae also have been formed in cultures where no nitrogen was included in the nutrient solution.

A small amount of evidence was obtained to show that nitrogen is readily assimilated by seedlings without mycorrhizae if the nitrogen is present in inorganic compounds, but that when the nitrogen is present only in organic compounds, especially complex proteins, the seedlings exhibit signs of nitrogen starvation. The presence of mycorrhizae did nothing to alleviate this apparent starvation. Indeed, no conclusive proof was obtained to show that the presence of mycorrhizae on the roots of seedlings is either helpful or harmful to the seedlings.

The formation of mycorrhizae depends on the contact of the right species of fungous mycelium with a growing root tip. No penetration of fungous hyphae was found in older roots. Elongation of a rootlet ceased upon formation of a fungous mantle around its tip. The fungus appeared to be immediately stimulated by contact with the root tip.

Attempts to form mycorrhizae with species of Penicillium, Rhizopus, and Mucor were unsuccessful.

Information was obtained concerning the nutrient and temperature requirements of several fungi which form mycorrhizae.

Fungi held for more than a year in artificial culture did not lose their ability to form mycorrhizae rapidly and in large numbers.

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CHROMOSOMES IN GRASS SORGHUMS[1]

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INTRODUCTION

Studies have been undertaken by several investigators to determine whether variations in chromosome number or abnormalities in chromosome behavior during reduction phases are associated with taxonomic differences, unusual genetic behavior, or unexpected results in plant-breeding experiments.

The present study of chromosome number in grass sorghums was suggested by the fact that perennial teosinte has twice as many chromosomes as the annual form and its close relative, corn (*Zea mays* L.).[2] It seemed of interest to examine the chromosome number in annual and perennial sorghums, since there are, for example, two species, *Sorghum sudanensis* (Piper) Stapf. and *S. halepensis* (L.) Pers., that are hardly distinguishable except that the former is an annual and the latter produces rhizomes and behaves as a perennial. This condition is almost a duplicate of that found in annual and perennial teosinte (*Euchlaena mexicana* Schrad. and *E. perennis* Hitchc.). It seemed possible that a chromosome study of these two and other sorghums might show a difference similar to that found in teosinte.

Plants of 10 different sorghum species were grown in the greenhouses at Washington.[3] The cytological preparations were made from fresh material stained with iron-aceto-carmine solution. The pollen mother cells were collected while undergoing the reduction division phases. One species, *S. versicolor* Anderss., was also studied after the anthers were killed, embedded, sectioned, and stained with Haidenhain's haematoxylin.

JOHNSON GRASS

Sorghum halepensis (Johnson grass) sends off rhizomes from the crown which live over winter and start up as new plants the following spring. This species has 20 bivalent chromosomes which are favorably distributed for counting in the prophase of the first reduction division. Figure 1, D, shows clearly the 20 paired chromosomes of *S. halepensis*. Prophases of the two daughter cells of *S. halepensis* showing 20 chromosomes in each cell are illustrated in Figure 1, E.

ANNUAL SORGHUMS

Seven species of sorghum that do not put out rhizomes from the crown and normally live only one season were found to have 10 bivalent chromosomes in prophases of the first division of the pollen mother cells.

[1] Received for publication July 28, 1931; issued April, 1932.

[2] LONGLEY, A. E. CHROMOSOMES IN MAIZE AND MAIZE RELATIVES. Jour. Agr. Research 28: 673-682, illus. 1924.

[3] H. N. Vinall, of the Division of Forage Crops and Diseases, supplied the seed of the sorghums studied and cooperated in the work as it progressed.

Sorghum sudanensis, the Sudan grass of commerce, is an annual and has only 10 chromosomes, in contrast to the 20 found in the very similar perennial Johnson grass. Three reduction phases of *S. sudanensis* are shown in Figure 1, A–C. The chromosomes of six other annuals are shown in Figure 2. A, B, and C show first-division metaphase, anaphase, and early telophase, respectively, of *S. verticilliflorum* (Steud.) Stapf., a wild grass sorghum very common in South Africa, where it is known as Tabucki grass. This species also has the haploid chromosome number 10.

Characteristic spindles of the first-division late metaphase and anaphase of *Sorghum virgatum* are shown in Figure 2, D and E. The 10 chromosomes divide almost simultaneously and the halves move

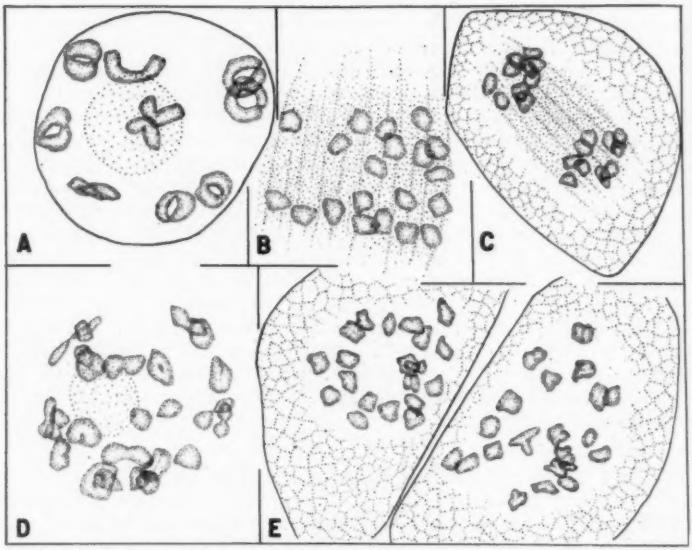

FIGURE 1.—Chromosomes in pollen mother cells of *Sorghum sudanensis* and *S. halepensis*, × 1,500: A, B, and C, Diakinesis, first-division anaphase and second-division anaphase, respectively, in *S. sudanensis;* D and E, diakinesis and second-division prophase, respectively, in *S. halepensis*

regularly to the two poles. Figure 2, F, shows the 10 bivalent chromosomes of *S. drummondii* at a time when rather characteristic differences are apparent. G is a later phase, showing a side view of the 10 chromosomes as they divide. I and J show the 10 bivalent chromosomes of *S. hewisonii* and *S. arundinaceum*, respectively. A second-division metaphase of *S. arundinaceum* is illustrated in K. An end view of the chromosomes of both cells is shown. More frequently the preparations show the two spindles at right angles, a position that is not so favorable for determining the number of chromosomes in the two daughter cells.

An eighth annual sorghum species, *Sorghum versicolor*, a short-lived annual coming from southeastern Africa, was found to have five as its haploid chromosome number. Figure 3, A–F, shows six characteristic phases in the reduction divisions of the developing pollen mother cells

of this species. Material from this species was unusually favorable material for chromosome study; the chromosomes are large, the number small, and many of the second-division spindles showed the five V-shaped chromosomes going to the poles in an almost stereotyped manner.

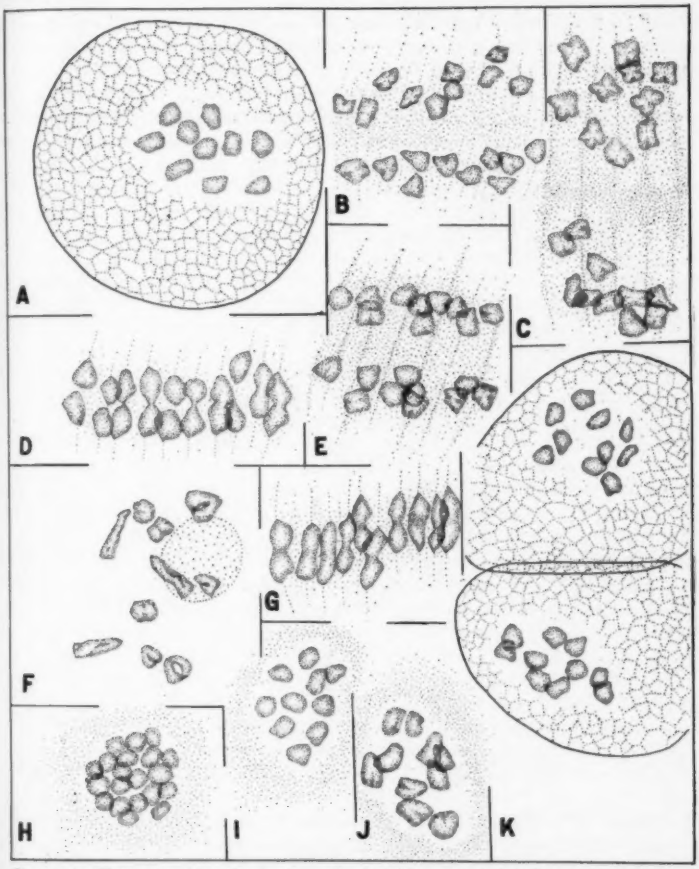

FIGURE 2.—Chromosomes in pollen mother cells of sorghums, × 1,500: A, B, and C, first-division phases in *Sorghum verticilliflorum;* D and E, first-division metaphase and anaphase in *S. virgatum* (Hack.) Stapf.; F and G, diakinesis and first-division metaphase in *S. drummondii* Nees; H, first-division metaphase in *S. purpureo-sericeum* (Hachst.) Aschers. and Schweinf.; I, first-division metaphase in *S. hewisonii;* J, first-division metaphase in *S. arundinaceum* (Willd.) Stapf.; K, second-division metaphase in *S. arundinaceum*

The haploid chromosome number of *Sorghum purpureo-sericeum* was found to be 20, as seen in diakinesis of the pollen mother cells. Figure 2, H, shows a typical first-division metaphase in which 20 chromosomes can be readily counted. This annual representative of the genus Sorghum is an exception to the usual chromosome condition in this genus, in which, with this exception, all annual forms were found to have 10 or less as their haploid chromosome number.

DISCUSSION

THE GENUS SORGHUM

The outstanding result of this determination of chromosome numbers in a few representative grass sorghums is that the perennial Johnson grass has double the number of chromosomes found in the annuals. This difference between Johnson grass and Sudan grass in chromosome number was also found in other annual sorghum species available for study. In all but two a haploid chromosome number of 10 was found in the annual forms and double this number in the perennial form. The two exceptions are *Sorghum purpureo-sericeum*, which has 20 chromosomes, and *S. versicolor*, which has only five.

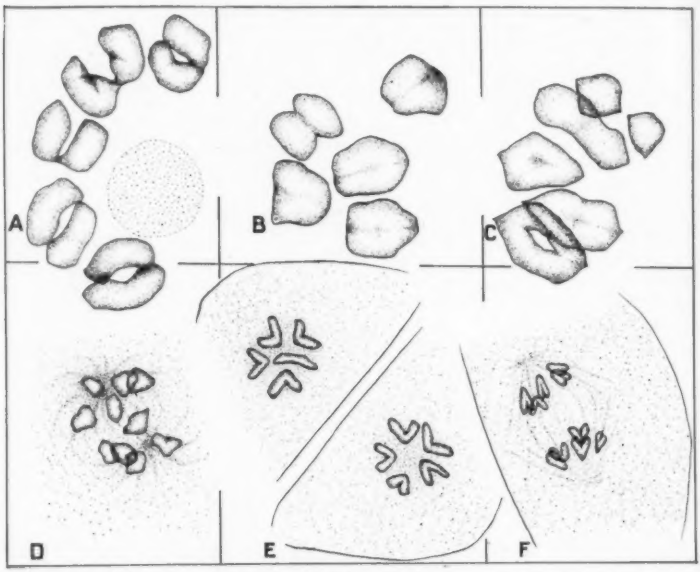

FIGURE 3.—Division phases in pollen mother cells of *Sorghum versicolor:* A, Diakinesis; B and C, first-division metaphases, early and late; D, first-division anaphase; E, second-division meta phase; F, second-division anaphase. (A, B, and C, × 1,500; D, E, and F, × 1,000)

It is probably a coincidence that the decrease in chromosome number is usually found associated with a reduction in life span. *S. versicolor* is a shorter-lived annual than any of the forms with 10 or more chromosomes.

Kuwada [4] was the first to study the chromosomes in sorghum. He found that *S. vulgare* Pers. has 10 as its reduced number. Marchal [5] found the same number for this species, and the writer found no deviation from this number in any of the following varieties: Blackhull kafir, Dwarf Shantung kaoliang, feterita, Sumac sorgo, White kaoliang, Black Amber sorgo, Dwarf Yellow milo, and Dwarf White milo.

[4] KUWADA Y. ÜBER DIE CHROMOSOMENZAHL VON ZEA MAYS L. Bot. Mag. [Tokyo] 29: 83-89, illus. 1915.
———— DIE CHROMOSOMENZAHL VON ZEA MAYS L. Jour. Col. Sci., Imp. Univ. Tokyo 39, art. 10, 148 p., illus. 1919.

[5] MARCHAL, E. J. J. RECHERCHES SUR LES VARIATIONS NUMÉRIQUES DES CHROMOSOMES DANS LA SÉRIE VÉGÉTALE. 108 p., illus. Bruxelles. [1920.]

The habit of this species is that of an annual, and the chromosome number is that found in most of the annual grass sorghums.

SORGHASTRUM NUTANS, CLOSELY RELATED TO SORGHUM

Sorghastrum nutans (L.) Nash is a species that taxonomically is not far removed from the sorghums. Church [6] has found 20 to be the reduced chromosome number of this perennial grass, which is the number found in perennial sorghum species.

OTHER GENERA OF ANDROPOGONEAE

Among those genera of the tribe Andropogoneae which are more distantly related to sorghum the analogy in chromosome numbers is not so close. In Erianthus, Bremer [7] found 30 to be the haploid chromosome number of four species. *Miscanthus sinensis* var. *zebrinus* Beal was found by Church [8] to have 21 chromosomes in gametic cells. *Andropogon ischaemum* L., according to Kuwada,[9] has 34, and *A. scoparius* Michx. and *A. furcatus* Muhl. have, respectively, 55/2 and 34, according to Church, as their haploid chromosome numbers. In the genus Saccharum the lowest chromosome number reported is that of *S. narenga* (Ham.) Wall., which Bremer gives as 15. Other species and species hybrids with chromosome numbers ranging from 20 to more than 100 have been studied by Kuwada and by Bremer. All plants of these five genera are perennials, and the lowest chromosome number found was 15; so that nothing contradictory to the relationship between high chromosome number and perennial habit is encountered in this group.

Exceptions similar to that found in *Sorghum purpureo-sericeum* were found in species from the more distantly related genera. These exceptions are *Imperata cylindrica* (Lam.) Beauv., which Bremer [10] found had 10 as its haploid chromosome number, and *Cymbopogon nardus* (L.) Rendle, in which Kuwada [11] found this same low number. Both of these species are perennial, and unless there exist related annual species with five chromosomes, the relationship found between high chromosome number and the perennial character is not a rule that applies to all genera of the tribe Andropogoneae.

The association between low chromosome number and the annual habit finds but one exception among the sorghum species and only a few exceptions in the closer relatives that have been examined cytologically. The chromosome condition is identical with that found in Euchlaena and its relatives, in which the perennial species has double the chromosome number of the annual form.

CONCLUSION

If the chromosomes of the perennial sorghums represent a duplication of the chromosome set found in annual forms, as seems to be the case in perennial teosinte, it indicates that the perennial plants have been derived from annual ancestors having 10 chromosomes. Whether this be the case or not, chromosome number is a character that should be of considerable value in the classification of the sorghums.

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[6] CHURCH, G. L. MEIOTIC PHENOMENA IN CERTAIN GRAMINEAE, II. PANICEAE AND ANDROPOGONEAE. Bot. Gaz. 88: 63-84, illus. 1929.
[7] BREMER, G. THE CYTOLOGY OF THE SUGARCANE. Genetica 7: [293]-322, illus. 1925.
[8] CHURCH, G. L. Op. cit.
[9] KUWADA, Y. Op. cit. (See footnote 4, second citation.)
[10] BREMER, G. Op. cit.
[11] KUWADA, Y. Op. cit. (See footnote 4, second citation.)

THE GROWTH CURVE IN BARLEY[1]

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INTRODUCTION

When an investigation of the catalase activity in relation to the growth of barley was initiated it was found to involve a comprehensive study of the growth curve of the plant. The results of the growth studies proved to be of sufficient interest to warrant a separate paper on this aspect of the original problem. A second paper deals specifically with catalase activity in relation to the growth curve (*17*).[3]

MATERIAL AND METHODS

Plants on which growth measurements were taken were grown at the Arlington Experiment Farm, Rosslyn, Va. Two varieties of barley were sown: Hannchen, C. I.[4] No. 531, a 2-rowed spring type that stools abundantly; and Tennessee Winter, C. I. No. 3546, a 6-rowed variety that stools less freely. One plot of each variety was sown on March 14, 1928. The soil in these plots was fairly good but was subject to considerable erosion.

Daily samplings for growth measurements were begun with Hannchen on March 28 and with Tennessee Winter on March 30 and were continued to June 4. After this date the interval between samplings was lengthened to two or more days because of the shortage of suitable material. At the beginning the daily samples, consisting of 20 plants, were all taken from one end of the plot, working toward the other end on succeeding days. The plots soon showed considerable variability; so the most nearly uniform part of each plot was divided transversely into six parts of approximately equal area, and the same number of plants were taken, as they came, from each division to make up the daily samples. On April 17 variability was further reduced by thinning the plants 2 to 3 inches apart in the drill row and by rejecting abnormally small ones.

Since it is impossible to obtain all the roots of a field-grown barley plant, the present investigation was made upon that portion of the plant above the seed. Later, when the permanent roots appeared, the portion of the stem above them was used. The tops varied in weight with the number of tillers per plant; so the main-shoot measurements only were made the basis of this study, as the main shoots varied less than the entire plants. The variation in the weights of individual plants as compared with that in the weights of their main shoots is shown in Figure 1.

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[1] Received for publication July 16, 1931; issued April, 1932. This paper is adapted from a dissertation submitted to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer acknowledges his indebtedness to Dr. C. O. Appleman, of the University of Maryland, for many helpful suggestions and criticisms.

[3] Reference is made by number (italic) to Literature Cited, p. 340.

[4] C. I. No. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

The plants were dug or pulled, wrapped in moist cheesecloth, and taken to the laboratory. The main shoots were then removed, placed again in moist cheesecloth, and kept there until they were measured and weighed. Abnormally small plants without vigorous tillers were discarded. The length in centimeters of the main shoot from seed or crown to the extreme leaf tip and the green weight of the main shoot were determined for each plant. While the plants were small, representative shoots were bulked and dried to constant weight in a vacuum oven at 80° C. The average dry weight per plant was then calculated from the average green weight. As the plants became larger all the main shoots in a day's sample were clipped into approximately ¾-inch pieces and mixed thoroughly. Determinations

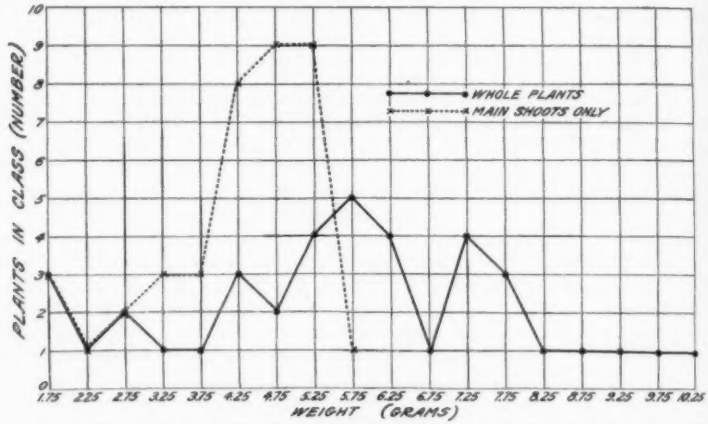

FIGURE 1.—Frequency distribution comparing the variability in green weight of whole plants with that of their main shoots. (Data from greenhouse population of a single day)

of dry matter were made on duplicate 5-g.[5] samples, or even on 10-g.[5] samples when the plants became still larger.

By the eighty-fifth day most of the kernels had started to develop. Beginning on this day separate measurements of both length and weight were made on the culm and the spike. These measurements made possible a study of the growth of the shoot exclusive of the kernels.

Notes were also taken on the condition of reserve foods in seed endosperm, leaf stage, presence and stage of development of seminal and permanent roots, presence of tillers, appearance of boot leaf, emergence and length of awns, emergence of spike, color and dehiscence of anthers, age of developing kernel, browning of awn tips, browning of glumes, and cessation of translocation in the kernels as shown by degree of drying.

FACTORS AFFECTING RELIABILITY OF THE DATA

An ideal experiment on growth would be one conducted under temperature and moisture conditions exactly optimum at all times. This study was made upon plants grown under conditions of about normal temperature and slightly subnormal but sufficient rainfall. On the whole the climatic conditions were rather favorable for spring-sown

[5] g is the abbreviation recently adopted by the Government Printing Office for grams.

barley. Variations in amount of growth from the ideal may be expected and when present are reflected in the curve of growth. A different succession of weather changes would produce a slightly different curve, as is indicated by the work of Gregory (*5*), who, in studying the effect of environment on the growth of barley, found a high positive correlation between day temperature and both net assimilation rate and efficiency index, and a significantly positive correlation between day temperature and relative leaf growth. The correlation between night temperature and these three indicators of growth was significantly negative in each case. Relative leaf growth was highly and negatively correlated with radiation, and efficiency index was independent of radiation.

Total height, or the length from crown to farthest leaf-tip extension, indicates fairly well the growth stage of a plant. As a measure of growth it is, however, subject to certain sources of error. The leaves of a grass are alternate, and growth occurs in the basal region of the leaf. Each leaf has its own grand period of growth, and therefore the grand period of growth, as indicated by the curve of the height of the plant, varies with the growing activity of the leaf extending the greatest distance distally from the crown. The younger leaf will be past its early slow-growing stage when it pushes out beyond the tip of the next older leaf which has just about finished its period of rapid growth. If the height of a single plant is measured at intervals short enough, such as are shown in Figure 2, the curve of growth will show a succession of accelerations corresponding to the appearance of the tip of young, actively growing leaves. This error is greatly reduced by averaging the measurements of a plant population which varies in number and stage of growing leaves. It is clearly present, however, in the height-growth curves at about the time of endosperm exhaustion. At this time the seedlings were very nearly in the same stage, the first leaf nearly at maximum length and the second leaf, while actively growing, needing several days' growth to equal the first in length.

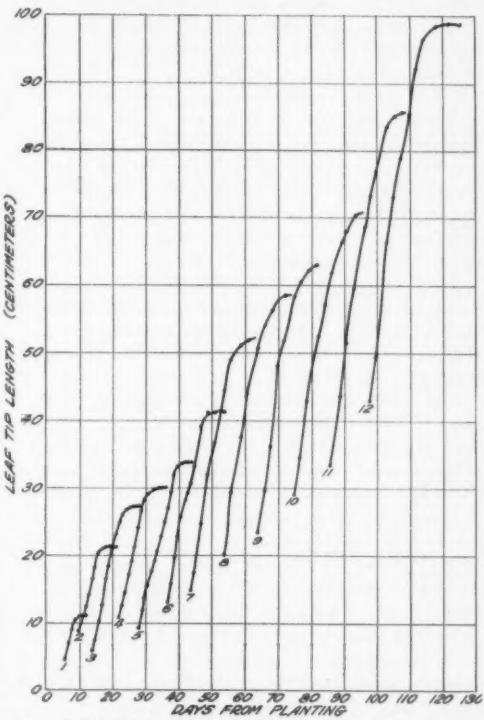

FIGURE 2.—Length-growth curve of plant No. 50, Arlington greenhouse, 1930, showing measurements of each successive leaf from its appearance until elongation ceased

Another source of error is the fact that neither the absolute nor the relative height of a plant variety is constant for different environmental conditions. This was shown by Harlan (7). Abyssinian barley was tall at Chico, Calif., and very short at St. Paul, Minn., Williston, N. Dak., and Moccasin, Mont. Odessa barley was tall at Williston and Moccasin and very short at St. Paul, and stood eighth on the list of 13 varieties at Chico. This error, due to varietal variation, is at least partially overcome by sowing the two types of barley.

The use of green weight in measuring growth also is subject to several errors. At the stage characterized by rapid increase of dry matter there may be an actual loss of water, causing the green weight to remain approximately stationary. As the plant nears maturity the moisture content decreases and the green weight approaches the dry weight in value. At this stage green weights indicate little or no growth, when actually that process is progressing rapidly. Green weight during active growth also varies according to the tissue turgidity, which in turn depends on the available soil moisture and the air moisture. Small unremoved drops of rain or dew on the plant at sampling time also introduce error.

Deposition, or at least translocation, of solids in the plant continues until growth ceases and the plant parts die or become dormant. Dry weight is the most reliable measure of growth and is emphasized in this study. Even this standard is not, however, free from error. At all stages of development and growth, parts of the plant are liable to mechanical and pathological injuries which are not always recognizable. This is especially true during the maturing phases, when the leaves gradually die; they are also leached by rain and may be broken off by wind. The situation is still further complicated by the fact that from flowering to maturity the developing seeds (which are new plants parasitic on the parent) were weighed with the spike, since it was impracticable to separate the grain from the seed coats, the latter being maternal tissues which it is practically impossible to remove from the caryopsis. An approximation of the dry weight of the shoot without the seeds was reached by adding to the average dry weight of the culm the average dry weight of the spike on the day of flowering. This is recognized as only an approximation but is considered rather close, as the growth of the maternal tissues in the spike probably is very nearly completed at this stage.

WEATHER CONDITIONS DURING THE GROWING PERIOD

Mean daily temperatures for the period from March 1 to July 8, 1928, computed with the aid of the planimeter from thermograph records, are shown in Figure 3, and amount and distribution of rainfall in Figure 4. A summary of the weather conditions for the same period in Washington, 2 miles away, is given in Table 1.

The cool weather, especially in June, allowed an almost normal development of the plants. This cool weather, together with a slight deficiency in rainfall, was unfavorable to such plant diseases as are caused by Helminthosporium and rust, which often result in serious injury to spring-sown grains in Virginia.

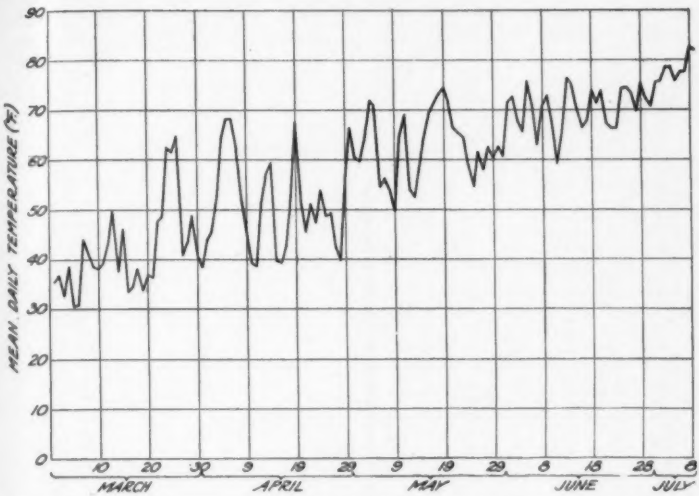

FIGURE 3.—Mean daily temperature (in ° F.) from March 1 to July 8, 1928, at the Arlington Experiment Farm, Rosslyn, Va.

TABLE 1.—*Summary of weather conditions from March 1 to July 9, 1928, at Washington, D. C.*

Item	March	April	May	June	July (1–9)
Dates of killing frosts	19, 20, 31	16, 17, 18	(a)	(a)	(a)
Accumulated excess (+) or deficiency (−) in temperature since first day of month ° F.	+49	−42	−23	−40	+28
Excess (+) or deficiency (−) in precipitation this month as compared with normal inches	−1.58	+1.25	+0.31	−1.47	−1.16
Accumulated excess (+) or deficiency (−) since Jan. 1 inches	−2.82	−1.57	−1.26	−2.47	−3.63
Mean percentage of relative humidity:					
8 a. m.	74	67	74	79	72
Local noon	55	49	51	62	52
8 p. m.	54	54	60	70	63
Normal percentage of relative humidity:					
8 a. m.	68	68	69	74	[b] 78
Local noon	51	48	50	56	[b] 57
8 p. m.	55	54	58	66	[b] 73
Mean percentage sunshine	60	55	62	53	85
Normal percentage sunshine	51	58	61	62	[b] 64
Total hours possible sunshine	371.1	397.4	444.0	446.0	133.3

[a] None. [b] For the month.

DEVELOPMENTAL STAGES OF THE BARLEY PLANT

Morphologically there are certain definite stages in the development of the barley plant. These stages and the plant age at which they occurred in this study are noted in Table 2. The placing of these stages in the growth curve should aid in interpreting the latter.

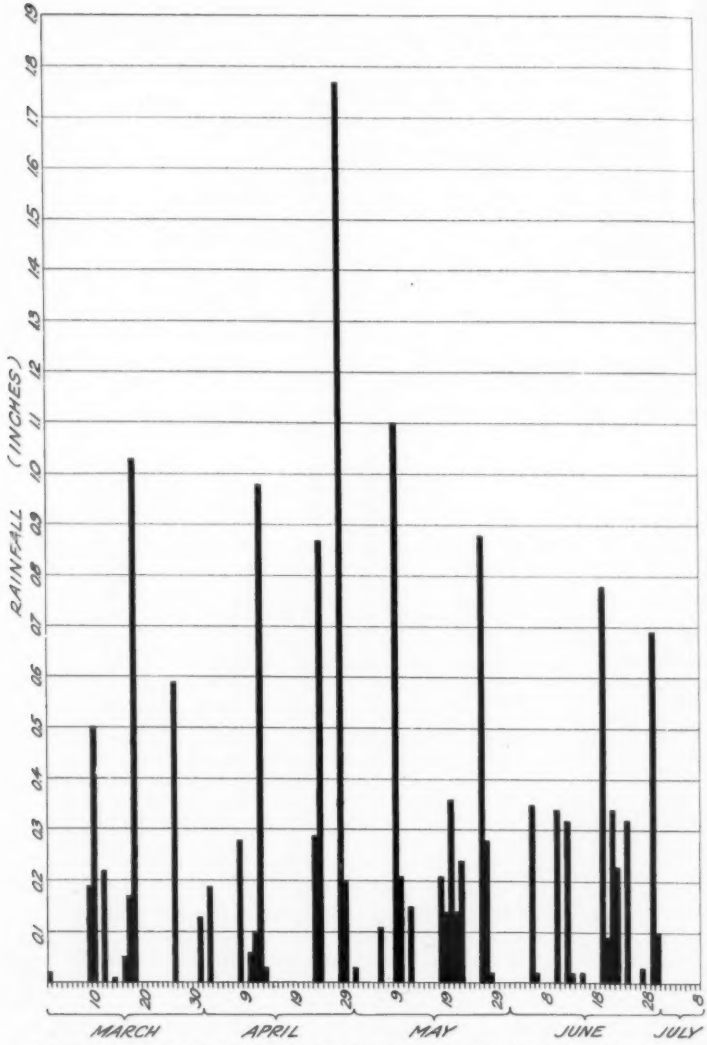

FIGURE 4.—Rainfall (in inches) from March 1 to July 8, 1928, at the Arlington Experiment Farm, Rosslyn, Va.

TABLE 2.—*Morphological stages of development in the barley plant, and the plant age at which these stages occurred at the Arlington Experiment Farm, Rosslyn, Va., in 1928*

Stages of development	Hannchen variety		Tennessee Winter variety (spring-sown)	
	Date	Plant age	Date	Plant age
		Days		*Days*
First leaf	Mar. 29	15	Mar. 31	17
Second leaf	Apr. 5	22		
Endosperm nearly exhausted	Apr. 8	25	Apr. 5	22
Scutellum shriveled	Apr. 10	27	Apr. 7	24
Third leaf	Apr. 16	33	Apr. 17	34
Crown well defined	Apr. 17	34	do	34
Crown roots started	do	34		
Tillers first appear	Apr. 22	39	Apr. 20	37
Fourth leaf	Apr. 24	41	Apr. 21	38
Tillers well developed	May 2	49	May 2	49
Jointing	May 18	65	May 18	65
Basal leaves mostly dead	May 21	68	May 21	68
Boot leaf showing	May 31	78	May 30	77
First awns emerging	June 2	80	June 2	80
First flowering	June 7	85	June 7	85
Tip of boot leaf dying	June 17	95		
Awns brown at tip			June 19	97
Spikes nearly dried out	July 2	110	July 2	110

EXPERIMENTAL DATA

GROWTH IN LENGTH

The length-growth curves of the two varieties, Tennessee Winter and Hannchen, are given in Figures 5 and 6. These curves show that the first leaf of the plants grew rapidly in length up to April 8, when the endosperm was almost exhausted. Growth of the crown roots was first evident on April 17, coincident with a second increase in rapid elongation. It is true that this second increase followed a 3-day period of relatively high temperatures, but from a study of the large-scale graph (fig. 9) of the dry weights of the two varieties, it would seem that temperature as a factor might be disregarded, since Hannchen showed an inflection in the curve at this time.

About April 21 there began another period of slower growth. The temperatures during this period were neither very high nor very low. First tillers had appeared but had not yet developed good roots. By April 21 the tiller roots in Hannchen were developing well and the curve again started upward. This increase, however, again was accompanied by higher temperatures, so that here, too, it is difficult to determine whether the increase in growth rate was due to additional root tissue or to higher temperatures. Leaf length began to taper off rapidly in Tennessee Winter on June 7, when first flowering occurred, the awn-tip length tapering off on June 17. In Hannchen flowering occurred on June 7, but the leaf length tapered off much more slowly, and the awn-tip length grew rapidly until June 17.

With both the barley varieties used in these experiments the results differ from those of Van de Sande-Bakhuyzen and Alsberg (*21*) with wheat, who found an inflection of the curve of length occurring at flowering. One factor contributing to this difference may be that wheat flowers at a later stage of elongation than does barley.

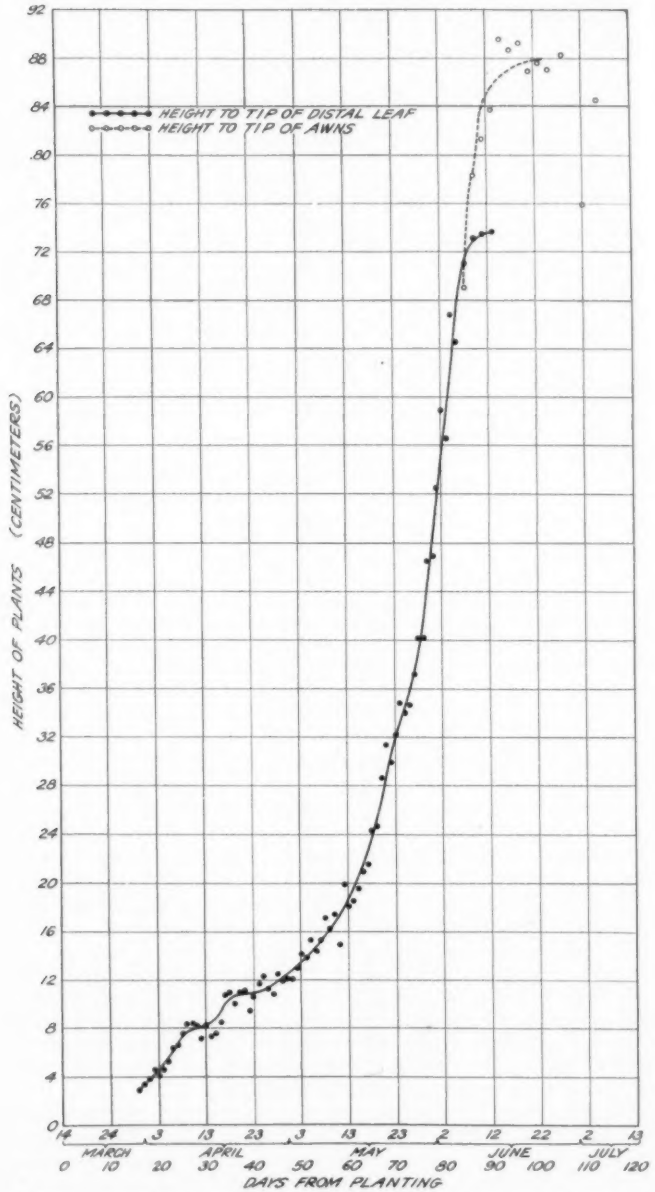

FIGURE 5.—Growth in length of spring-sown Tennessee Winter barley from emergence to maturity

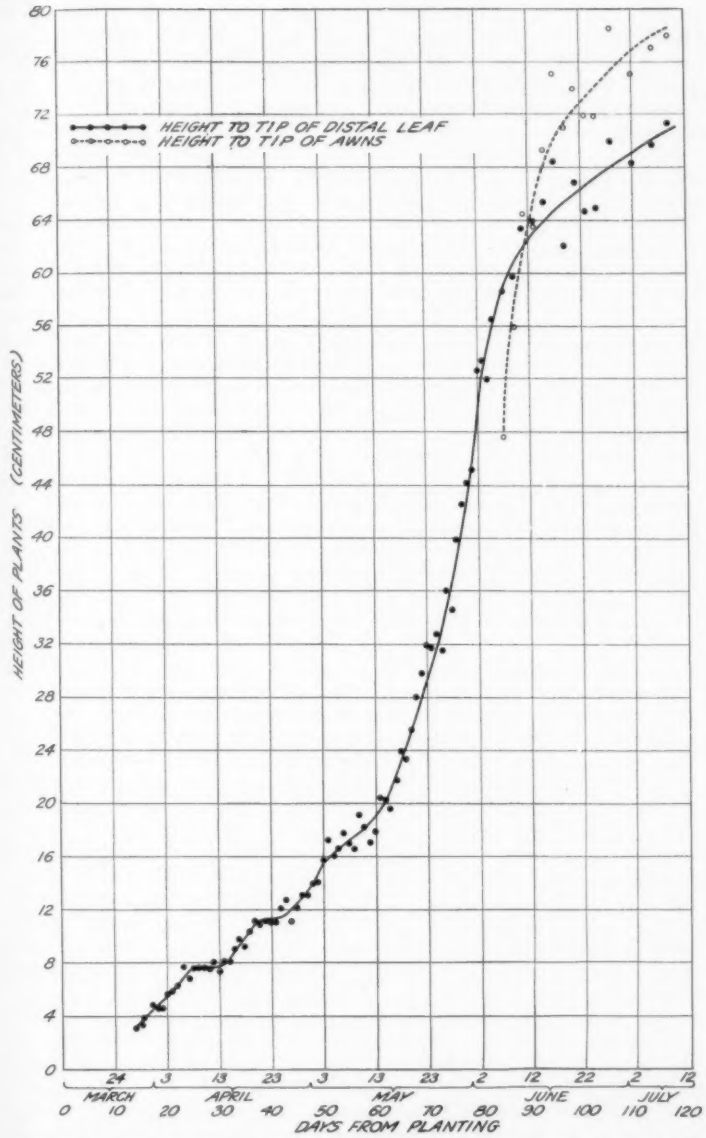

FIGURE 6.—Growth in length of spring-sown Hannchen barley from emergence to maturity

GROWTH IN WEIGHT

The daily growth in weight of Tennessee Winter is shown in Figure 7. This curve is comparatively smooth. Prior to May 2 the curves of both green and dry weights had risen only slightly above the base line. During this period length-growth rate of the shoot varied with stage of development much more than did weight-growth rate. However, a larger scale graph (fig. 9) shows a tapering off in weight-growth rate as well as in length-growth rate coincident with exhaustion of the endosperm (second-leaf stage). Throughout this early period there is evident a gradual increase in weight-growth rate, which reached its maximum on May 24, shortly after jointing began.

First flowering occurred on June 7, and after this date the green and dry weights of culm and spike were determined separately. In order to correct for kernel weight, the dry weight of the spike of June 7 was added to the dry weight of the culm on each of the days following that date. The sum of these two weights was the weight of the shoot. This procedure is believed to be justified, since the spike tissue, excluding the kernels, had very nearly reached its maximum mass on June 7. Any error occurring probably would be on the side of less weight.

It will be seen from the curve that when weights of the shoots minus the kernels are plotted as a continuation of the shoot curve before June 7, no perceptible inflection occurs until June 11. This again fails to agree with the results of Van de Sande-Bakhuyzen and Alsberg (*21*) for the length-growth curve of wheat. The weight of the spike alone started an abrupt increase on June 7, as would be expected owing to the rapid increase in kernel mass. The weight of the culm alone increased at an equally rapid rate until June 11, tapered off slightly on June 13, and after a drop on June 15 climbed again until June 19. After this date leaching by rain and leaf breakage caused a decided drop. As was found by Burd (*2*), the total weight of the shoot plus kernels increased with almost no change in the direction of the curve to June 19 (97 days), when the awns commenced to turn brown at the tips. From this time on the plants rapidly turned brown and the kernels finally dried out, being almost dry and waxy on July 2.

The growth in weight of Hannchen (fig. 8) differed somewhat from that of Tennessee Winter. In the early part of the growth period (up to April 7) Hannchen was generally heavier in both green and dry weights; from April 8 to 16 it was almost the same; but from April 18 to maturity it was outstripped by Tennessee Winter. Final green and dry weights were much smaller for Hannchen. However, similar changes in the growth curve, as shown in Figure 9, occurred at very nearly the same points as in Tennessee Winter. There was an increase in rate of growth after May 2, when tillering became active, and a further acceleration beginning about May 22, shortly after jointing had been initiated.

In determining the developmental stage of the plant a rough average of 20 plants was used. Temperature as a factor in modifying the curves in the period from April 17 to May 3 may be disregarded, since the inflections were initiated on different dates in the two varieties (fig. 9) and seem to have no relation to a variation in that period of about 29° F. in mean daily temperature. In order to hook up the stage of development more definitely with the growth curve in this early period of growth, the curve of early weight growth in Hannchen

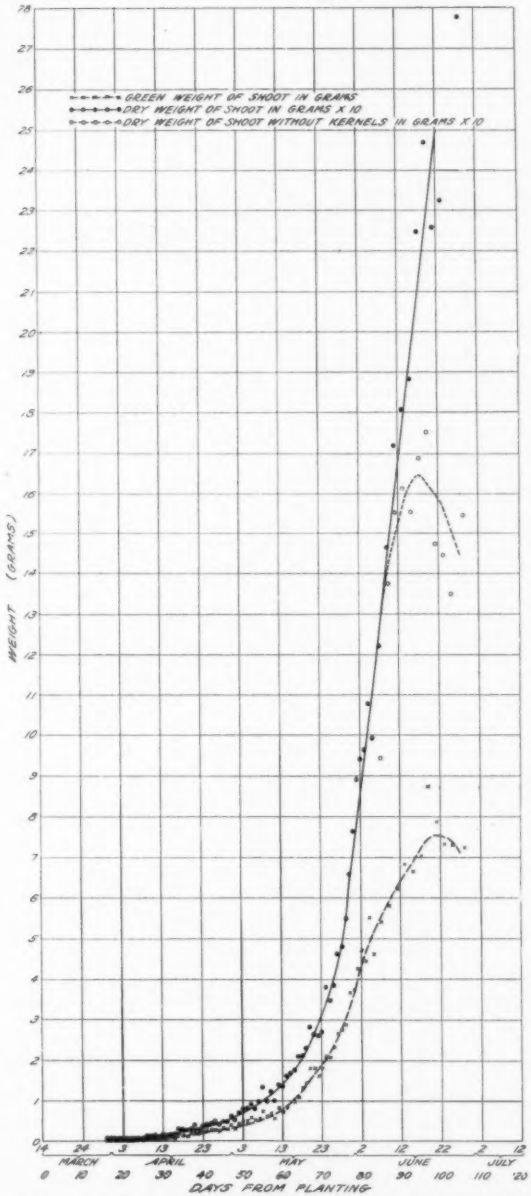

FIGURE 7.—Growth in average green weight of main shoot, dry weight of main shoot, and dry weight of main shoot, with kernels excluded, of spring-sown Tennessee Winter barley from emergence to maturity

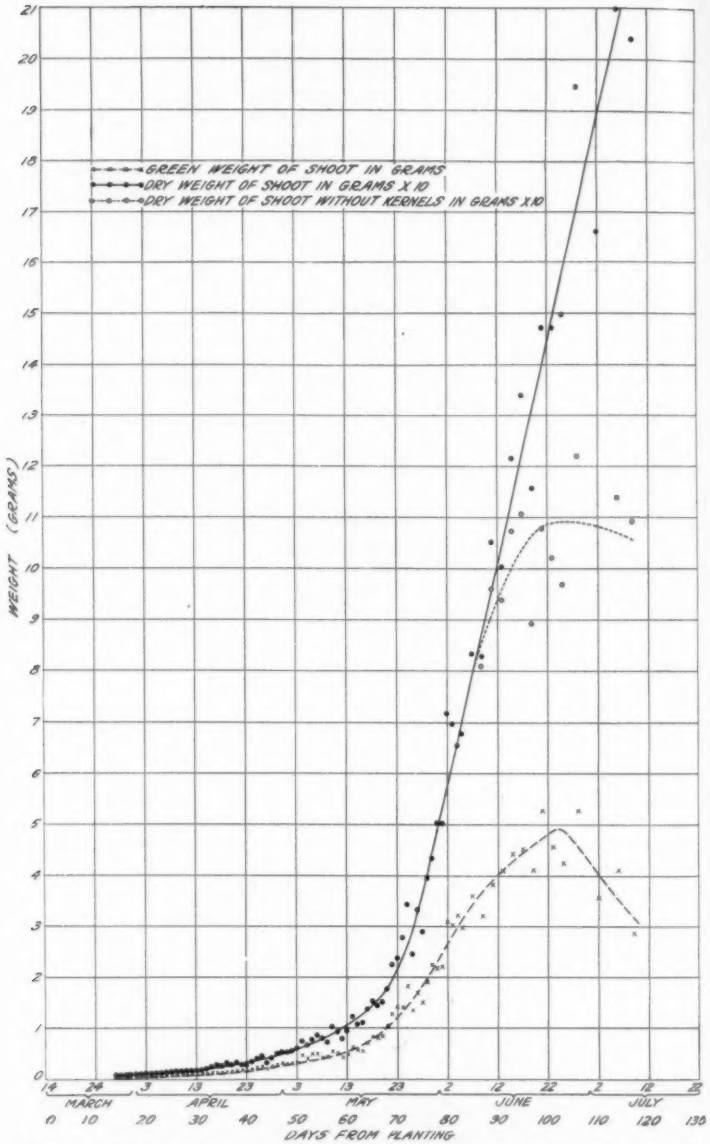
GREEN WEIGHT OF SHOOT IN GRAMS
DRY WEIGHT OF SHOOT IN GRAMS X 10
DRY WEIGHT OF SHOOT WITHOUT KERNELS IN GRAMS X 10
WEIGHT (GRAMS)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
14 24 3 13 23 3 13 23 2 12 22 2 12 22
MARCH APRIL MAY JUNE JULY
0 10 20 30 40 50 60 70 80 90 100 110 120 130
DAYS FROM PLANTING

FIGURE 8.—Growth in average green weight of main shoot and in dry weight of main shoot with kernels excluded of spring-sown Hannchen barley from emergence to maturity

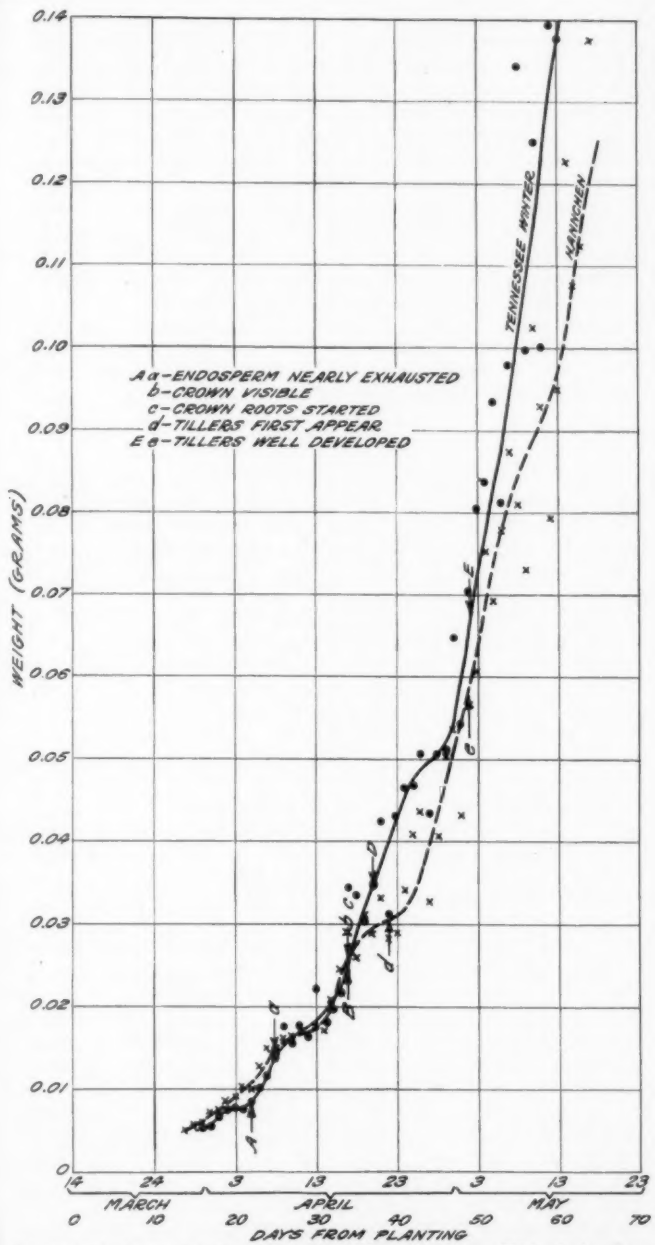

FIGURE 9.—Early growth in dry weight of Hannchen and Tennessee Winter barley shown on a larger scale than in Figures 7 and 8

barley was again determined daily from 10 plant samples grown in the greenhouse at the Arlington farm during the winter of 1930–31. (Fig. 10.) The ranges and variations in temperature were fairly uniform from day to day and may be disregarded. On the eighteenth day tillers were first large enough to be detached and weighed separately. Two days later secondary subcrown roots had appeared, and slightly exceeded 5 mm in length. These roots became functional the next day. Evidently this accession of root tissue was insufficient to supply nutrients to both main shoot and tillers, for while the growth rate of the plant was maintained almost without change, the rate of main-shoot growth slowed up materially until the thirty-eighth day, when all plants first acquired functional crown roots and their main shoots attained a new maximum growth rate.

Like Tennessee Winter, Hannchen (fig. 8) shows no perceptible inflection of the weight-growth curve of the shoot without the kernels for a number of days. The slight inflection on June 15 becomes marked on June 20, about 12 days after flowering.

DISCUSSION

A number of investigators *(1, 10, 11, 15, 16, 18)* have shown the existence of correlations between portions of the growth curve and definite stages in the development of the plant. Some *(4, 6, 11, 13, 14, 16, 19, 21)* believe that the water or nutrient supply is the limiting factor in certain growth stages, or that competition for this material occurs between different parts of the plant.

The data herein presented indicate that lack of sufficient root tissue is a factor that inhibits increase in dry weight in the barley plant before crown roots begin to function. Another flattening of the dry-weight curve, which is even more apparent in the length curve, occurs at the time of rapid tiller development and is accompanied by enhanced growth of crown roots. The development and growth of the spike primordium into the full-sized flowering head is accompanied by a rapid elongation and increase in dry weight of the whole shoot. Up to the beginning of this period of rapid elongation ("jointing" or "shooting") the amount of absorptive tissue evidently has been a factor limiting the growth of the plant. At the time of most rapid tiller growth these young parts, growing much more actively than the main shoot, may compete successfully for the nutrients. The rapid growth of the tillers is accompanied by an increase in root growth until the tillers are nearly the size of the main shoot, when there is sufficient root tissue to supply both. Active jointing then follows. Another factor now enters to complicate the process. In order to maintain an upright posture the jointing shoot must be strong enough not only to support its own weight but successfully to resist wind action and at maturity to carry a heavy load of starch-laden seeds. This condition is met by the deposition of seemingly inactive materials, such as cellulose, lignin, silica, etc., in the culm. This process, of course, rapidly takes much tissue out of the realm of active metabolism, thereby slowing down the rate of growth. There is for some time, however, enough actively growing tissue to more than counterbalance this loss. But barley is a plant of determinate growth. Normally there is no branching at the upper nodes with the exception of the modified branches and leaves which constitute the floral organs in the

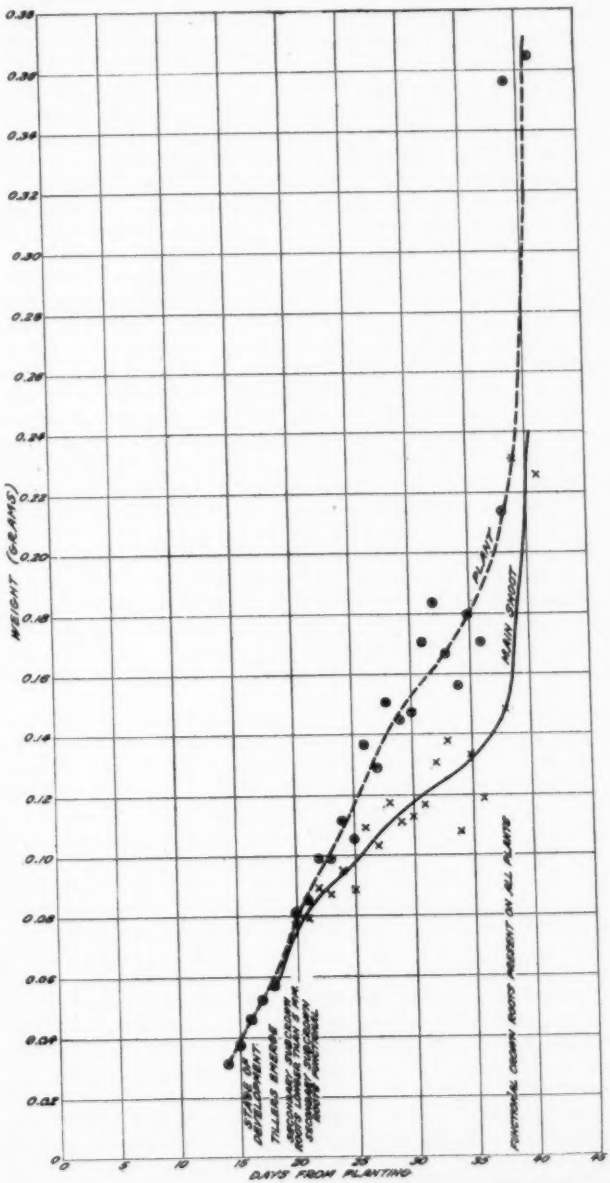

FIGURE 10.—Early growth in dry weight of Hannchen barley, greenhouse grown

spike. The flowering head is the terminus of the shoot. If this does not proliferate, length growth must cease and the inflection of the curve of awn-tip length will be the inflection of the curve of shoot growth.

In contrast to the findings of Van de Sande-Bakhuyzen and Alsberg (*21*) with wheat, there is in barley no major inflection in total length or in total dry weight at flowering. There is, however, an inflection of the leaf-length curve at or shortly after flowering. This latter, however, does not affect the height of the plant. The growth habits of a plant must be considered in making such generalizations. Both maize and wheat extrude their flower heads above the leaf tips before flowering takes place. At anthesis the upward growth is about ready to stop. It is very different with barley, where flowering generally occurs while the spike is still inclosed in the boot leaf. The last internode of the culm bearing the spike is still young and composed of soft growing tissue and continues to elongate until the spike emerges from the sheath and extends, in most varieties, above the end of the distal culm leaf. The basal leaves are, for the most part, dead at the time jointing begins, and necrosis proceeds distally, first appearing in the leaf tips and working back through the blade to the leaf sheath. The top or boot leaf begins to die at the tip when the kernels are about one-half or two-thirds their mature age, and the awn tips begin to brown a few days later. The culm axis or stalk loses its chlorophyll rapidly at this time. However, the leaf sheaths protect the stalk from excessive drying for a considerable time and the vessels are still capable of conducting the soil solution to the still actively growing kernels. After the chlorophyllous tissue has ceased to function there probably is still translocation of dry matter from the upper part of the stalk and the spike into the kernels even after the shoot has been harvested. This certainly is true when the kernels are immature when harvested (*9*).

If the growth in dry weight is considered, there is found a state of affairs entirely different from growth in length. Not only does the inflection of the curve not occur at flowering, but there seems to be no true inflection at all. This is due to the fact that the growing seeds present in the spike serve as storage organs for an amount of dry matter which may equal or exceed the total dry matter contained in the remainder of the shoot. The deposition of this material begins actively a few days after fertilization of the egg cell and continues for nearly a month, when structural difficulties abruptly halt growth (*8*).

It seems to the writer that events occurring at the time of root formation, together with the acceptance of the compound-interest principle, may form the basis of a hypothesis applicable to the whole growth curve of plants. *Postulating favorable environmental conditions, rate of growth in the plant is largely, if not entirely, conditioned by the maximum nutritional opportunities in different parts of the plant and the ability of the plant from a physical and chemical standpoint to take advantage of these opportunities.*

According to this hypothesis the aerial portions of the young plant will grow with increasing rapidity according to the compound-interest formula, provided the roots have an absorbing and carrying capacity sufficient to satisfy the nutritional needs of the plant. These "maximum nutritional opportunities" are greatest during the middle of the growth period, where the compound-interest formula best fits the

curve, as is shown by its regular and steep ascent. As plant parts mature, with the accumulation of inactive substances such as cellulose and lignin, there is relatively less tissue capable of growth. Such inactive tissue is no longer "principal," and "principal" increases more slowly or at a rate directly proportional to the actual amount of actively growing tissue. If a sufficient amount of actively growing tissue is maintained, the growth rate will continue constant. Such a condition exists in plants of indeterminate growth, such as the tomato. Here increase in size continues until the production of organs, such as flowers and fruits, destroys the requisite nutritional balance for active growth of the vegetative growing point. In a plant of determinate growth, such as barley, where branching of the shoot does not occur normally and the spike is produced at the apex, growth continues in the culm and spike until inactive materials are laid down in all parts in sufficient amount to hinder enlargement. Growth will then stop.

In the barley kernels growth limitation is largely due to starch congestion. Such large amounts of this material are stored in the endosperm cells that mitotic division ceases, and under favorable conditions all the available cell space is filled with starch. This has been demonstrated by Cobb (*3*) in wheat. This deposition of starch in amounts sufficiently large to hinder cell division places a limit on the maximum size of the kernel. Starch deposition proceeds until prevented by drying out, lack of necessary materials, or the tension of the cell walls.

The slackening of top growth during root formation, as noted by Pearsall (*16*) and Priestley and Evershed (*18*), probably is due, in part at least, to the inability of available roots to absorb nutrients in sufficient amount to balance the photosynthetic activity possible in the leaves and stem.

Any disappearance of water from the plant at flowering, as reported by Van de Sande-Bakhuyzen (*20*), would seem to be due merely to deposition of inactive material and consequent drying out of tissues that are approaching senescence. However, Hurd-Karrer and Taylor (*12, p. 397*) found in their work with wheat that "the physiological processes involved in flowering did not exert any specific effect on their water content."

SUMMARY AND CONCLUSIONS

The growth curve of the barley shoot without kernels is typically sigmoid, with certain slight variations from a perfectly smooth curve in both early and late stages. Such a curve is a composite picture of the grand periods of growth of all the organs and also of each individual cell in the measured individual.

Variations in the curve of early growth are associated with definite events in the development of the plant. The hypothesis that the structural inability of the existing roots to absorb nutrients is the factor inhibiting growth in length is supported by the fact that this inhibition is removed as soon as new roots are formed and become functional.

Growth rate in both length and weight is retarded at about the time first tillers are appearing and before their roots are established. When tiller roots become functional the curve rises more sharply.

The curve of leaf length reaches inflection at about first flowering, but the curves of awn-tip length and total length of shoot reach inflec-

tion 5 days later in the Tennessee Winter variety and 8 to 10 days later in Hannchen.

The curve of shoot weight, excluding kernels, shows inflection 4 days after flowering in Tennessee Winter and 10 to 12 days after flowering in Hannchen.

The total weight of the shoot, including kernels, increases at a regular rate until about the hard-dough stage of the kernel, when the leaves and awns have begun to die.

After the hard-dough stage the shoot loses weight on account of mechanical breakage and leaching by rain.

Indirect evidence indicates that growth rate in the plant varies with the accessibility of nutrients and the structural ability of the plant to translocate them and utilize them in growth.

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CATALASE ACTIVITY IN RELATION TO THE GROWTH CURVE IN BARLEY [1]

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INTRODUCTION

Phenomena associated with growth might be expected to exhibit a grand period paralleling that of growth itself. Catalase activity is well-nigh universal in living, growing tissue. It has been found to be correlated with various phases of metabolism, but its nature and function are problematical. During a study of the growth curve of barley a parallel investigation was made upon catalase activity. The results are reported in this paper.

REVIEW OF LITERATURE

Weiss and Harvey (*14*)[3] found catalase activity to be strongly correlated with growth in the proliferation produced by the potato-wart disease in spite of the high acidity of the growing tissues.

Heinicke (*8*) found not only that there was more catalase activity in the leaves of apple trees fertilized with sodium nitrate than in those of the checks, but also that when only half the plant was given nitrate the portion directly above gave an increased catalase reaction. This indicated that the unfertilized half of the plant could be used as a check. Heinicke suggests that, since catalase activity shows the nitrogen effect in the apple when chemical analysis gives no significant difference in nitrogen content, "it is very probable that the ability to decompose hydrogen peroxide is a more sensitive measure of the metabolic status of the tissue than the usual chemical analysis." He makes an interesting inference when he says:

> Many of the preparations of apple-leaf tissue show greater power to decompose hydrogen peroxide than is reported in the literature for tissue from organs more actively engaged in growth processes.

Auchter (*2*) confirmed the findings of Heinicke on apple-leaf tissue and found that catalase activity was greatly increased in the leaves whenever nitrate of soda was applied to plants of privet, oak, and peach. In all these the growth became more vigorous and the plants contained a significantly greater amount of nitrogen than did the check plants to which no nitrate was added.

Shull and Davis (*13*) state that in the dimorphic seeds of Xanthium, the upper seed, which shows a delayed germination, exhibits constantly less catalase per unit of dry matter than does the lower seed.

Rhine (*12*) believes that catalase activity is somehow related to the presence of oxygen and oxidation. She found, however, that during

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[2] The writer acknowledges his indebtedness to Dr. C. O. Appleman, of the University of Maryland, for many helpful suggestions and criticisms.

[3] Reference is made by number (italic) to Literature Cited, p 355.

germination as respiration rapidly increased catalase activity decreased, and inferred that the latter can not be a part of the respiratory mechanism. She concludes that catalase activity can be a measure of metabolism only when there is no rapid change in respiration.

Knott (*10*) found that the youngest and oldest leaves of spinach usually are low in catalase activity, while those intermediate in age have higher and approximately equal activity. His results are open to the criticism that the young leaves probably contained a much lower dry-matter content, which should be corrected for, and the oldest leaves were yellowing and dying.

Ezell and Crist (*6*) found a significantly negative correlation between catalase activity and average weight of 65 lots of lettuce plants, and Haber (*7*) found that "catalase activity, growth, and yield were negatively correlated in the vegetatively mature leaves, green-mature fruit, and ripe fruit" in the tomato.

There is much better agreement among plant physiologists than among animal physiologists. Becht's (*3*) results seem to indicate that the catalase reaction is unreliable as a measure of metabolism in animals. He found a variation of as much as 1,000 per cent in the blood of different dogs under "identical conditions." Morgulis (*11*) placed frogs under temperature conditions which "it was estimated" caused a change of 300 to 400 per cent in the metabolic rate, and found no accompanying influence on the catalase content of the frogs. He concluded that "it is certain that it [catalase activity] is certainly not a measure of metabolic activity."

Burge (*4*) and Burge and Burge (*5*) are convinced that catalase activity is definitely associated with metabolism and with respiration in particular.

MATERIAL AND METHODS

Preliminary experiments were run to determine the most nearly practicable optimum conditions for measuring catalase activity in the tissues studied. The methods so determined were used throughout the investigation.

The apparatus used (fig. 1) is a modification of the one described by Appleman (*1*). It consists of a square, wooden, motor-driven arm sliding through supports at either end and carrying a Bunzel tube (22 mm. inside diameter), each arm of which has ample capacity for 4 c. c. of liquid. A flexible rubber tube of sufficient length to allow a full excursion of the shaker arm connects the Bunzel tube with a small-bore glass tube. This glass tube is in direct connection through a 3-way glass stopcock with the upper end of a 50 c. c. burette, the lower end of which is connected by a thick-wall rubber tube with the lower end of a second burette of the same capacity which may be raised or lowered at will to equalize the water levels in the two burettes. The gas-conducting portions of the apparatus are purposely made of small-bore material to reduce the volume of gas subject to temperature and pressure changes. No attempt was made to determine the volume at normal temperature and pressure, because, as noted later, the error from this source is less than that from other variable, uncontrolled factors. The Bunzel tube is immersed in a water thermostat electrically heated and controlled. A knife-type heater is used, and the thermoregulator is of the mercury type, sensitive to about $\pm 0.02^{\circ}$ C., working through a mercury relay in which both poles are perma-

nently bathed in mercury. The water temperature is equalized by a motor-driven stirrer of the turbine type. The Bunzel tube is shaken at the rate of approximately 204 complete excursions per minute, and the thermostat is kept constant at 24.5°. In starting the shaker motor the switch is thrown one second before the minute, which allows the first mixture to occur approximately on the minute.

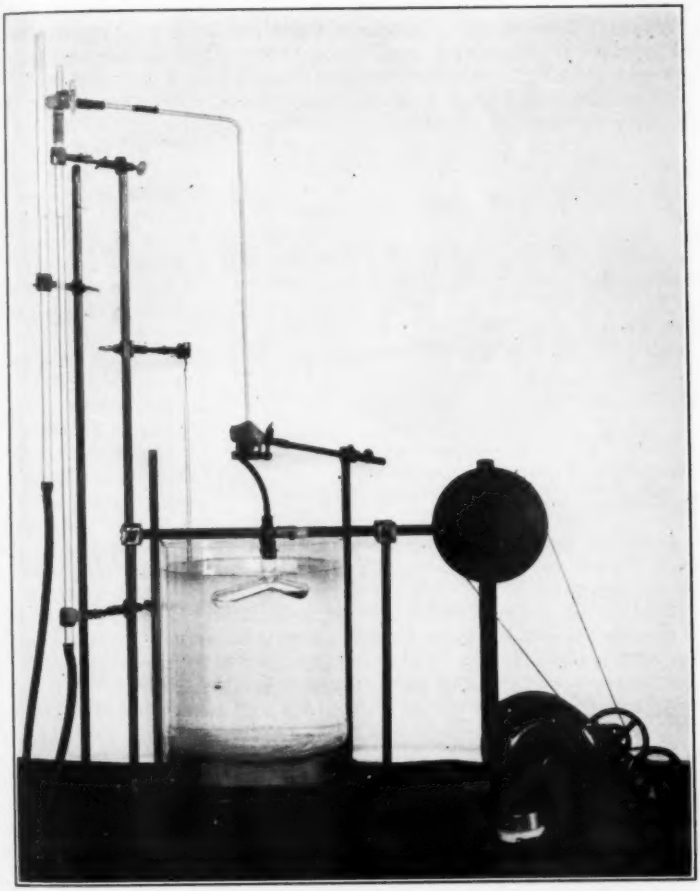


FIGURE 1.—Apparatus for the determination of catalase activity (without thermostatic equipment)

Readings are taken at the end of each successive minute for periods of 3, 5, or 10 minutes.

Determinations of catalase activity were made in the following way: The main shoots of five plants of Hannchen spring barley (C. I.[4] No. 531), selected at random from the 20 taken as a single day's

[4] C. I. refers to accession number of Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

sample for growth study, were clipped finely with shears and mixed thoroughly. From this composite a 5-g sample[5] was weighed out and quickly treated with powdered calcium carbonate, the cut ends being covered to prevent acidity from developing at those points. A small quantity of water and where necessary a little quartz sand that had been carefully cleaned and thoroughly washed were added and the mixture was reduced in the mortar to a thin paste. Distilled water[5] was then added to make 250 cm^3. The mixture was thoroughly stirred, and a 2 cm^3 sample, which contained 0.04 g of green tissue, was pipetted into one arm of a Bunzel tube. In the other arm of the tube was placed 2 cm^3 of 12-volume Dioxogen (hydrogen peroxide), and the tube was attached to the shaker cork in the thermostat and left three minutes to come to temperature equilibrium. The 3-way cock in the top of the burette was opened and water leveled to 0.0 cm^3. At the end of the 3-minute period the cock was turned so that passage from the Bunzel tube was open to the burette. One second before the minute the switch was thrown, and the shaking began very nearly on the minute. Gas immediately began to be evolved on the mixture of the hydrogen peroxide and the diluted plant material, and the displaced gas was collected in the burette. The water surfaces in the burette and the leveling tube were leveled off and the number of cubic centimeters of gas evolved was read, estimating to 0.01 cm^3, at the end of each minute for 10 successive minutes. Duplicate determinations were made. If the total amounts differed by as much as 1 cm^3, a third sample was run and the average of the three used. The average volume of gas evolved in 10 minutes was calculated for 0.008 g of dry matter, and this figure was taken as the "catalase activity" of the sample. This seems valid, as preliminary experiments have shown that through a rather wide range of dilutions catalase activity is proportional to the dilution. (Fig. 2.)

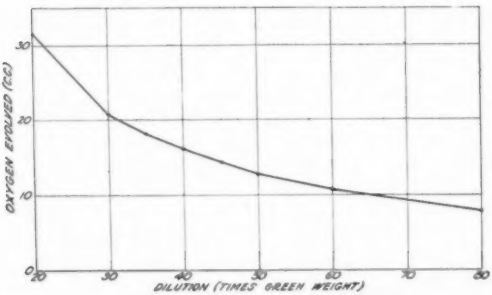


FIGURE 2.—Relation between the volume of oxygen liberated from hydrogen peroxide by 2 cm^3 of a tissue suspension and the dilution of that suspension

Dry matter determinations were made upon duplicate 5-gm. samples dried to constant weight at 80° C. in a vacuum of approximately 28 inches of mercury. After flowering, when the kernels in the spike began to grow rapidly, the shoot was divided into spike and culm and dried in separate samples. The percentage of dry matter for total shoots was figured from the two determinations.

Throughout the investigation care was taken to give all samples as nearly identical treatment as possible, and the results are believed to be consistent.

[5] g and cm^3 are the abbreviations recently adopted by the Government Printing Office for grams and cubic centimeter, respectively.

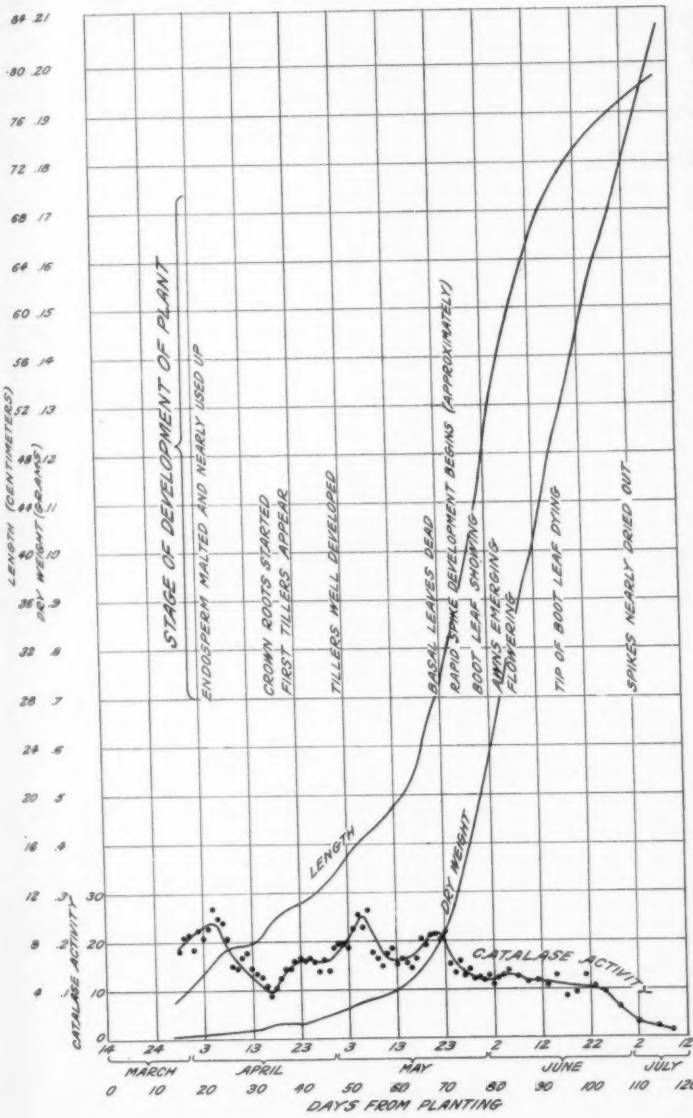


FIGURE 3.—Stage of development, growth in length, dry weight, and catalase activity per unit of dry weight of the main shoot of Hannchen barley from emergence to maturity, grown in plots at the Arlington Experiment Farm, Rosslyn, Va., 1928

CURVE OF CATALASE ACTIVITY IN THE BARLEY PLANT

In Table 1 are shown the following data for the main shoot of barley: Average length in centimeters, average dry weight in grams, catalase activity found in 0.04 g of green tissue, and the catalase activity calculated for 0.008 g of dry matter of the daily samples.

In Figure 3 the amount of catalase activity per unit of dry weight in the shoot of the Hannchen barley plant is plotted against time. On the same sheet are shown the curves of total length and total dry weight of main shoot. The stages of development of the plant on different dates also are indicated.

TABLE 1.—*Average length, average dry weight, and catalase activity in the main shoot of spring-sown Hannchen barley, 1928*

Day of harvest		Length	Dry Weight	Catalase activity (O_2 evolved in 10 minutes)	
Date	Days after planting			Per 0.04 gm. green tissue	Per 0.008 gm. dry matter
		Cm	*Grams*	*Cm³*	*Cm³*
Mar. 28	14	3.04	0.0051		
Mar. 29	15	3.29	.0056	17.93	17.99
Mar. 30	16	3.84	.0058	20.87	20.84
Mar. 31	17	4.70	.0073	21.51	21.03
Apr. 1	18	4.41	.0072	17.91	18.03
Apr. 2	19	4.58	.0086	20.54	22.02
Apr. 3	20	5.51	.0091	24.37	20.74
Apr. 4	21	5.76	.0101	23.51	22.42
Apr. 5	22	6.19	.0106	30.64	26.71
Apr. 6	23	7.65	.0128	25.46	24.61
Apr. 7	24	6.76	.0150	28.64	23.84
Apr. 8	25	7.43	.0148	19.21	20.06
Apr. 9	26	7.56	.0162	15.91	14.98
Apr. 10	27	7.67	.0164	13.29	14.22
Apr. 11	28	7.53	.0173	17.28	16.54
Apr. 12	29	8.11	.0164	17.26	17.42
Apr. 13	30	7.35	.0187	14.33	14.33
Apr. 14	31	8.14	.0184	13.32	13.43
Apr. 15	32	8.25	.0209	12.92	12.67
Apr. 16	33	9.08	.0245	11.32	10.95
Apr. 17	34	9.77	.0288	10.10	8.72
Apr. 18	35	9.25	.0262	10.73	10.21
Apr. 19	36	10.36	.0310	13.90	12.05
Apr. 20	37	11.16	.0290	12.20	14.24
Apr. 21	38	10.80	.0334	15.05	14.03
Apr. 22	39	11.23	.0284	13.35	15.93
Apr. 23	40	10.96	.0289	13.29	16.40
Apr. 24	41	11.00	.0342	14.18	15.73
Apr. 25	42	12.15	.0408	14.50	16.30
Apr. 26	43	12.77	.0437	13.40	15.64
Apr. 27	44	11.07	.0329	11.25	13.88
Apr. 28	45	12.19	.0406	14.06	16.00
Apr. 29	46	13.06	.0505	13.95	13.90
Apr. 30	47	13.07	.0535	17.07	18.45
May 1	48	14.09	.0531	16.82	19.31
May 2	49	14.16	.0568	16.89	19.14
May 3	50	15.70	.0606	16.72	18.61
May 4	51	17.29	.0751	17.74	22.48
May 5	52	16.11	.0692	21.80	25.29
May 6	53	16.58	.0779	18.12	22.76
May 7	54	17.84	.0873	22.63	26.34
May 8	55	16.98	.0809	16.47	17.45
May 9	56	16.47	.0731	13.50	16.20
May 10	57	19.15	.1024	13.93	14.91
May 11	58	18.33	.0927	15.50	17.16
May 12	59	17.09	.0792	16.65	18.14
May 13	60	17.91	.0950	14.75	15.28
May 14	61	20.39	.1224	15.64	16.21
May 15	62	20.24	.1075	14.23	15.46
May 16	63	19.56	.1123	14.16	14.35
May 17	64	21.77	.1373	15.73	16.08
May 18	65	23.94	.1533	18.65	20.33
May 19	66	23.31	.1453	17.15	19.19
May 20	67	25.55	.1516	18.91	21.09
May 21	68	28.04	.1781	17.96	21.20
May 22	69	29.79	.2258	17.57	20.21
May 23	70	31.92	.2387	18.24	21.79
May 24	71	31.70	.2789	14.93	15.13

TABLE 1.—*Average length, average dry weight, and catalase activity in the main shoot of spring-sown Hannchen barley, 1928*—Continued

Day of harvest		Length	Dry Weight	Catalase activity (O_2 evolved in 10 minutes)	
Date	Days after planting			Per 0.04 gm. green tissue	Per 0.008 gm. dry matter
		Cm	*Grams*	*Cm³*	*Cm³*
May 25	72	32.73	.3434	12.54	13.38
May 26	73	31.57	.2455	14.27	15.72
May 27	74	36.01	.3323	12.58	12.80
May 28	75	34.59	.2911	13.52	14.00
May 29	76	39.88	.3948	12.54	12.12
May 30	77	42.51	.4333	11.94	12.37
May 31	78	44.21	.5031	13.74	11.93
June 1	79	45.18	.5023	14.39	12.70
June 2	80	52.53	.7169	12.62	10.89
June 3	81	53.35	.6965	14.03	12.18
June 4	82	51.89	.6535	13.24	12.98
June 5	83	56.50	.6786	15.78	13.84
June 7	85	58.68	.8311	14.58	12.59
June 9	87	59.83	.8287	14.99	11.58
June 11	89	63.66	1.0519	16.38	11.88
June 13	91	64.09	1.0030	13.07	10.67
June 15	93	65.48	1.2159	17.86	12.93
June 17	95	68.56	1.3391	12.78	8.57
June 19	97	62.15	1.1565	13.00	9.18
June 21	99	66.99	1.4706	18.10	12.93
June 23	101	64.70	1.4705	16.89	10.43
June 25	103	64.89	1.4973	17.11	9.66
June 28	106	70.15	1.9455	11.93	6.34
July 2	110	68.50	1.6613	7.23	3.10
July 6	114	69.80	2.0970	5.76	2.24
July 9	117	71.47	2.0373	5.09	1.43

While there was a rather wide variation in catalase activity per unit (0.008 g) of dry weight from day to day in the 1928 data, there was a distinct trend apparent, as is indicated by the smoothed curve drawn through the points. There are four clearly defined elevations in the first 70 days of growth. During this time the plant was composed mainly of succulent actively growing tissue. The dry matter did not reach 20 per cent until the seventy-sixth day. After the seventieth day (May 23) the percentage of dry matter increased, gradually at first and then very rapidly as the seeds dried out. On May 23 the basal leaves were dead. The culm leaves soon died also, and on June 17 the boot leaf was dying, the maximum moisture remaining longest in the nodes and kernels. From May 23 the catalase activity decreased quite uniformly until June 25, when final drying out occurred and the catalase activity dropped to 1.43 cm^3 at the final determination. This drop in catalase activity is associated with rapid growth beginning about the seventieth day, the relation continuing unchanged up to the point where growth abruptly ceases.

Catalase determinations were run upon one planting only in 1928. To verify these data and to check their apparent relation to stage of plant growth, three supplementary series of determinations were run subsequently upon Hannchen barley. None of these latter, however, carried the plant to maturity. Only the early stages, where the earlier curve was more variable and consequently more subject to doubt, were studied in these later experiments. Figures 4, 5, and 6 show graphically the shoot length, dry weight of shoot, and catalase activity corrected for dry matter, together with the appearance of certain developmental stages in each of the three series. The first series was run upon material grown in the greenhouse in the late winter and spring of 1929.

This material was limited, and catalase determinations were not continued much beyond the time when the crown roots became functional. The curve in Figure 4 corresponds fairly well with that for the data previously obtained, except that the first low point does not occur so near the time of endosperm exhaustion as in the former.

The second series was run upon material grown in field plots in 1929. The curve in Figure 5 almost parallels that of the greenhouse material of the same year. On account of poor soil conditions the plants did not tiller well and appeared so abnormal that the experiment was discontinued on the fifty-second day. In the graph it is seen that from days 9 to 16 growth was rapid while the curve of catalase activity dropped. From days 17 to 24 growth lagged and an elevation in the catalase curve occurred. After day 24 rapid growth rate was again accompanied by a decreased catalase activity.

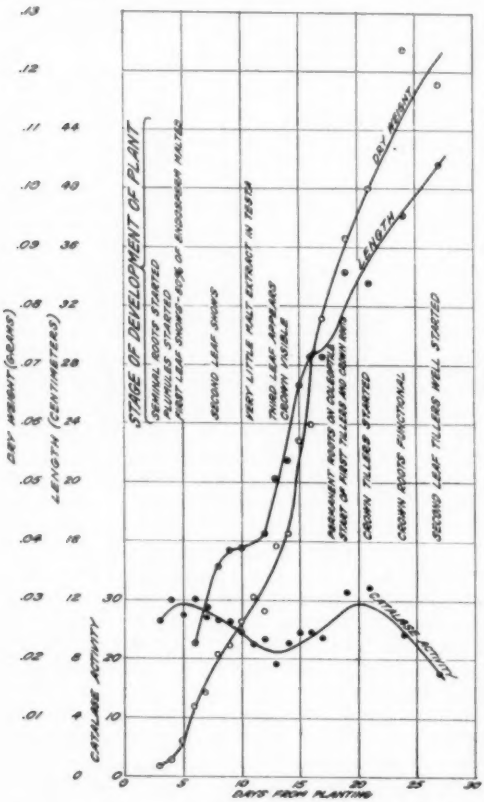
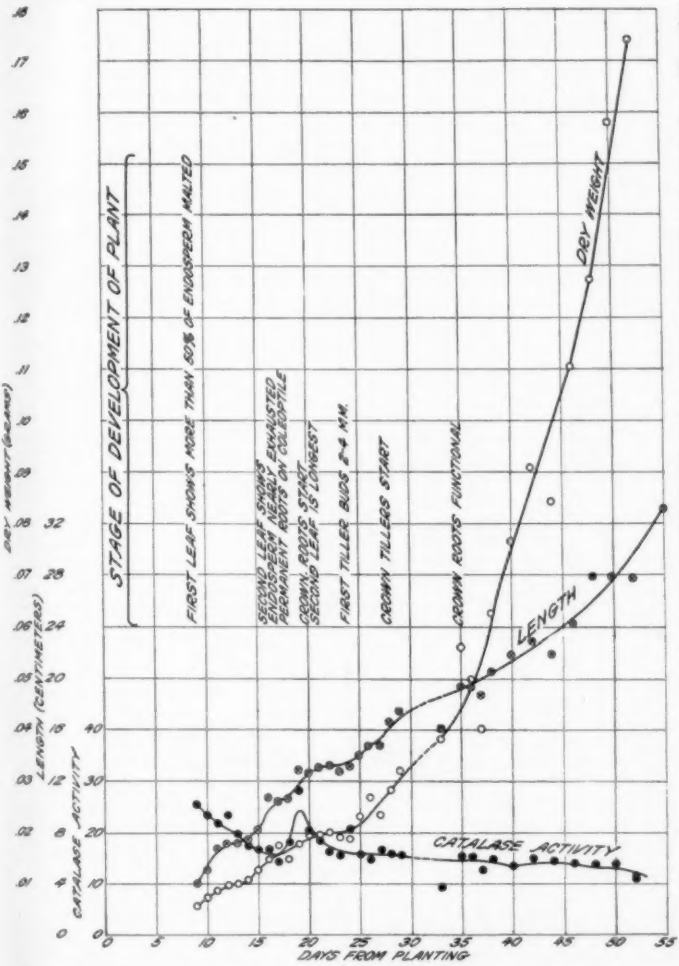


FIGURE 4.—Stage of development, growth in length, dry weight, and catalase activity per unit of dry weight of the main shoot of Hannchen barley grown in the greenhouse at the Arlington Experiment Farm, Rosslyn, Va., in the winter of 1928–29

The third series was run upon material grown in the greenhouse in the winter of 1929–30. Catalase determinations were made until jointing was well under way. In this series each determination was made upon the whole of the main shoot of a single plant, except in the early stages, when more material was desirable. The resulting curve (fig. 6) is reasonably smooth and resembles the other three quite closely. There is, however, no elevation during the time of active tiller production, as there is in the 1928 curve. The increase during jointing and immediately preceding the appearance of the floral organs, however, is significant and agrees with the 1928 data. Here also growth rate is rapid while catalase activity drops off. When growth lags the curve of catalase activity rises.

On account of environmental differences a definite stage of development naturally did not occur at the same age in days in all four experiments. In order to compare properly the curves in Figures 3, 4, 5, and 6, the data shown therein are plotted against the six definite developmental stages indicated.


STAGE OF DEVELOPMENT OF PLANT
FIRST LEAF SHOWS MORE THAN 50% OF ENDOSPERM MALTED
SECOND LEAF SHOWS ENDOSPERM NEARLY EXHAUSTED PERMANENT ROOTS ON COLEOPTILE
CROWN ROOTS START SECOND LEAF IS LONGEST
FIRST TILLER BUDS 4-5 MM.
CROWN TILLERS START
CROWN ROOTS FUNCTIONAL
DRY WEIGHT
LENGTH
CATALASE ACTIVITY
DRY WEIGHT (GRAMS)
LENGTH (CENTIMETERS)
CATALASE ACTIVITY
DAYS FROM PLANTING

URE 5.—Stage of development, growth in length, dry weight, and catalase activity per unit of dry weight of the main shoot of Hannchen barley grown in plots at the Arlington Experiment Farm, Rosslyn, Va., 1929

The resulting curves (fig. 7) are the ones already shown, lengthened, or shortened to fit the developmental stages indicated on the abscissa of each original curve. This makes possible a direct approximate comparison of the four series of determinations.

Considering the 1928 data alone, it seems possible to correlate increased intensity in the curve of catalase activity with the plant stages characterized by active metabolism. The data from the three subsequent series of determinations, however, indicate that so definite and inclusive a conclusion is not entirely justified. In all cases in 1928 (fig. 7) intensity of catalase activity is high in the germinating plant and gradually falls off during the rapid growth preceding the exhaustion of the endosperm. There then occurs a general slackening of

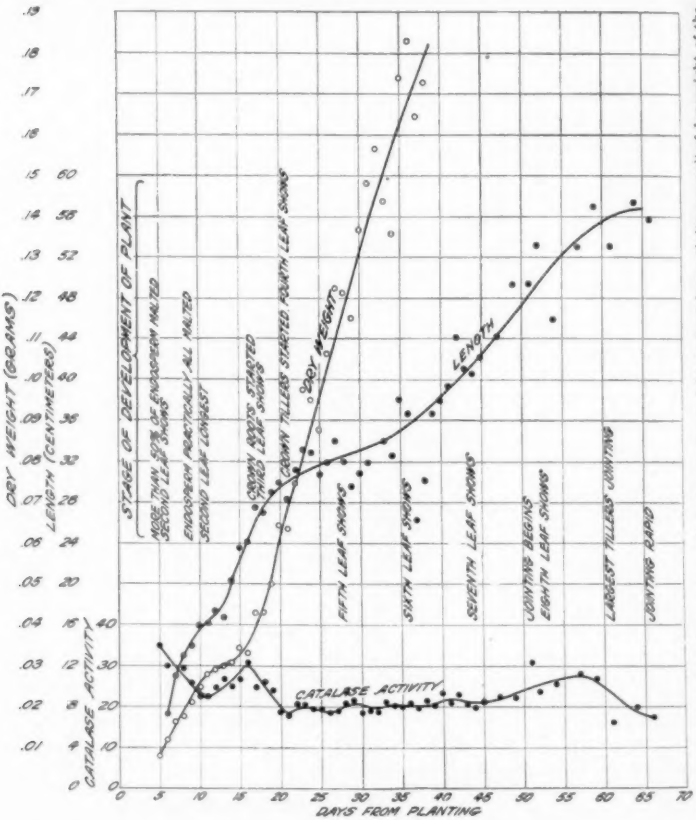


FIGURE 6.—Stage of development, growth in length, dry weight, and catalase activity per unit of dry weight of the main shoot of Hannchen barley grown in the greenhouse at the Arlington Experiment Farm, Rosslyn, Va., in the winter of 1929–30

growth rate, as indicated by dry-weight determinations. At the same time catalase activity per unit of dry matter begins to climb until the crown roots are functional, after which growth again becomes rapid and catalase activity decreases in intensity. There is evidence of another period of high catalase activity during jointing and just prior to the first appearance of the spike, with some indications of a high during the previous period of tiller production. As senility proceeds in the plant, catalase activity decreases and drops to a low level as the vegetative part of the plant dies.

DISCUSSION

The only measure of catalase activity is the volume of molecular oxygen liberated when the substance under investigation is brought into intimate contact with hydrogen peroxide. The presence of hydrogen peroxide in living tissue has not been proved. Consequently no assumptions can be made as to the nature of the reaction, its function, or its usefulness to the plant. Furthermore, if catalase activity be destroyed in normal tissue, as happens in determinations in the laboratory, no idea can be had of the total amount produced in the plant; the determinations then show merely the excess of manufacture over destruction.

In this study of catalase activity a composite sample of all the tissues of the entire main shoot was used. This was composed mainly of meristematic and young, actively growing tissues in the seedling stage. As age increased, the leaves rapidly matured, the stem was differentiated and became woody, and finally all the tissues except the seeds in the spike were in a dying condition. This course of events produces the typical sigmoid curve of growth. Had meristematic tissue alone been used for the daily catalase determinations, the curve

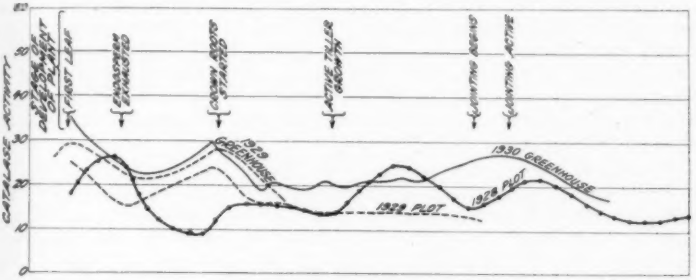


FIGURE 7.—Four series of catalase-activity determinations plotted against stage of development. (See figs. 3 to 6, inclusive)

probably would have differed as much from the one shown herein as a growth curve of meristematic tissue would differ from one constructed from entire-shoot data.

A low C/N ratio generally is accepted as necessary to active metabolism. Auchter's data (*2*) on woody plants indicate that an increase in carbohydrates in proportion to the nitrogen present is accompanied by a decreased catalase activity. Hicks (*9*) found in wheat the lowest C/N ratio in the distal end of an organ; for instance, the leaf tips have a lower C/N ratio than the bases even though the meristem of the leaves is at the base. From these results one would expect to find the greatest catalase activity in barley in tissues that are mature but not dying, and in plants that have a relatively large amount of mature living tissue. In the writer's determination of catalase activity in different parts of the barley plant [6] the mature portions seemed to have a higher catalase activity than did the very young tissues or those much past maturity; for instance, the flag of the boot leaf contained an amount greater than that of any other part tested, and the sheath of

[6] Unpublished data.

the boot leaf held second place in one variety and third in another, while the awns held third and second place, respectively. All the parts of the shoot, including the kernels, proximal to the awns were significantly lower in catalase activity.

It is evident that the total catalase activity in the plant approximately keeps pace with growth. But in order to make catalase exactly proportional to growth the curve of catalase activity (expressed as cubic centimeters of oxygen evolved per unit of dry matter) must be a straight line parallel with the horizontal axis. It will be seen that this condition does not obtain. There are certain elevations above the mean of the curve which must be explained. In all four series of determinations oxygen evolution is high at and immediately following germination, but drops off with active seedling growth and reaches a low point shortly after all the endosperm is malted. Growth then slackens somewhat, and catalase activity again climbs, reaching a second peak at the time of the appearance of the crown roots. Thereafter tiller buds appear and growth is accelerated in the plant and the curve drops to a level, which, in the 1930 greenhouse experiments, is maintained with but slight deviation until the peak during early jointing. In the 1928 plot material this peak during early jointing is preceded by another high point during active tiller growth, which does not appear in any other curve. During late jointing the spikes develop rapidly and catalase activity drops off (fig. 3) to a level which is maintained fairly constant until the actively growing portions of the plant are proportionately reduced by the deposition of a considerable amount of inactive structural material and the progressive death of the leaves from crown to spike.

From the foregoing it appears that the determinations of catalase activity are roughly proportional to the reciprocal of growth rate, being, in general, lowest during the stages of most active growth, as measured by length and by deposition of dry matter. Furthermore, three very definite elevations in the curve are evident: (1) During early germination; (2) during the development of the crown roots and before they become functional; and (3) during early jointing, which immediately precedes the appearance in the boot leaf of the floral spike. In other words, each peak in the curve occurs at the inception of a new and definite stage in the functional activity of the plant.

It is evident that the substance involved in catalase activity is produced during growth and that this production is, as has been intimated, approximately proportional to the amount of living tissue producing it. It is possible that the peaks in the curves may be explained by one of a number of assumptions. In the first place, it may be that the substance is produced at a uniform rate by the living cell, but is used up in the process of growth, and is more rapidly used up in rapid growth. In the second place, an old cell may be a larger producer than a young cell; consequently, during rapid active growth, when a proportionately large number of the cells are young, the average production would be lowered and the lagging production would result in a drop in the curve. In the third place, it might be assumed that relatively great physiological activity actually precedes rapid extension in length and deposition of dry matter.

SUMMARY AND CONCLUSIONS

Four series of daily determinations of catalase activity were made during the growth period of Hannchen barley in field plot and greenhouse. The first series was carried through to the mature plant; with the other three series the studies were continued through the period of early growth, where the results were most variable.

Catalase activity (i. e., cubic centimeters of oxygen gas liberated per unit of dry weight) is roughly proportional to the reciprocal of growth rate. Three hypothetical explanations are suggested.

The curve of catalase activity shows three peaks, each occurring at the time of inception of a new and definite stage in the functional activity of the plant.

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FACTORS INFLUENCING THE BLOOD-SUGAR LEVEL OF DAIRY CATTLE[1]

By R. E. HODGSON, formerly *Graduate Assistant*, W. H. RIDDELL, *Dairy Production Investigator*, and J. S. HUGHES, *Animal Nutritionist, Kansas Agricultural Experiment Station*

INTRODUCTION

The sugar of the blood of dairy animals has been the subject of considerable investigation in its relation to milk secretion and disease. It is the precursor of the lactose of milk. Meigs (*11*)[2] has presented a thorough review of the literature on this little-known phase of metabolism. There is good evidence to show that milk sugar is derived from the glucose of the blood, though the process by which the transformation takes place in the mammary gland remains obscure.

In recent years the behavior of the blood sugar has been studied in dairy cattle under certain abnormal conditions. Several investigators have presented figures on the normal concentration of this constituent. Hayden and Sholl (*5*) after 75 tests with 44 cows concluded that the average concentration of sugar in the blood of dairy cows is 51.75 milligrams per 100 cubic centimeters. Moussu and Moussu (*9*), working with only 10 cows of different breeds, gave a range of 0.061 to 0.08 per cent, while Hayden and Fish (*4*), as a result of 68 analyses, gave an average figure of 46.52 milligrams per 100 cubic centimeters with a range of 30 to 70 milligrams. In a later and more extensive piece of work, Hayden (*3*) reported the low average of 41.15 milligrams of sugar per 100 cubic centimeters of blood for 253 samples taken from 23 cows over a period of 11 months.

Scheicher (*12*), in studying the ratio of blood sugar to lactose in dairy cattle, observed that the sugar concentration of the blood ranged between 0.055 and 0.10 per cent and averaged 0.0744 per cent, while Anderson and his associates (*1*) in a recent investigation gave a range of 43.2 to 142.0 milligrams for animals of all ages and an average of 51.2 milligrams for animals in the older group.

It is evident from these results that the blood-sugar level of dairy animals, while tending toward a mean value somewhere near 50 milligrams per 100 cubic centimeters of blood, is subject to wide variations. The causes of these variations have not been discussed to any extent in articles reporting investigations in this field. Undoubtedly, however, they are due, in part, to the fact that the rate at which sugar enters the blood and the rate at which it leaves the blood vary under different conditions. The actual amount of sugar in the blood at any time depends on the relative intensity of these two opposing sets of conditions.

Figuratively speaking, there are three streams of sugar supplying the blood. One enters from the intestines, another arises from the hydrolysis of glycogen, and a third from the synthesis of sugar from

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[2] Reference is made by number (italic) to Literature Cited, p. 364.

other compounds. The first of these can be controlled to a considerable extent by limiting the amount and kind of carbohydrates in the feed. In the case of dairy animals where the digestion is slow and more or less continuous, the rate of absorption of monosaccharides from the digestive tract under ordinary conditions probably does not vary sufficiently to produce any marked fluctuation in the blood-sugar level. The second stream, namely, that arising from the hydrolysis of glycogen, is largely under the control of the nervous system. When an animal is excited, the rate of conversion of glycogen into glucose is increased through the action of the adrenal glands. Excitement may produce a pronounced increase in the sugar level of the blood in a short time. The third source of sugar, namely, the synthesis of sugar from other compounds, is controlled by the processes of metabolism. This source of sugar probably produces very little fluctuation in the blood-sugar level under ordinary conditions.

Sugar is being steadily withdrawn from the blood and oxidized to furnish energy for maintaining the various functions of the body. The amount varies greatly, depending on the degree of muscular activity. Sugar is also removed from the blood to provide the lactose in the milk of lactating animals. Under normal conditions any sugar in excess of that needed for the production of energy and lactose is stored as glycogen or converted into fat, in which form it is stored as reserve energy.

A knowledge of sugar metabolism and the factors that affect the blood-sugar level is important in studying the problem of milk secretion. It was in part for the purpose of securing such information that the experiments described in this paper were undertaken.

EXPERIMENTAL METHODS

One hundred and forty animals were used in these studies. All belonged to the college herd and were maintained under normal conditions of herd management. They were fed at 6 a. m. and 4 p. m. each day, and milked at 5 a. m. and 4 p. m.

The animals were selected and handled in such a way that information could be obtained concerning the influence of the following factors on the blood-sugar concentration: Age, breed, lactation, fasting, introduction of relatively large amounts of glucose into the stomach, and excitement. In many cases a single determination was used in more than one comparison. In studying the influence of any one factor, care was taken to see that other conditions affecting the blood-sugar level were maintained as nearly constant as possible.

In recent investigations (*6*, *10*) it has been shown that the various methods now in use for the determination of blood sugar may yield somewhat different results. Folin's new micro method (*2*), which appears to give results in close agreement with the actual sugar content, and which requires only 0.1 cubic centimeter of blood for each determination, was used in this work. The blood was drawn from the ear by means of a capillary pipette and rinsed into a 10 per cent sodium tungstate solution. In all cases the samples were centrifuged and prepared for analysis within one hour after the blood was drawn. The majority of the samples were collected at approximately 9 a. m.

FACTORS STUDIED

AGE

The data collected comprise 20 observations made on not less than eight animals in each of 11 age intervals. Animals of the four major dairy breeds were used. The mean blood-sugar values were calculated, and the results are assembled in Table 1.

TABLE 1.—*Influence of age on the blood sugar* [1] *of dairy cattle*

Age class of animals (inclusive)	Mean value for blood sugar [2]	Age class of animals (inclusive)	Mean value for blood sugar [2]
	Mg per 100 cm³		*Mg per 100 cm³*
1 to 6 days	100.4±1.685	16 to 19 months	62.2±1.000
1 to 4 weeks	88.2±1.226	20 to 23 months	55.0± .685
1 to 3 months	80.2±1.212	24 to 47 months	54.6± .770
4 to 7 months	75.4± .794	48 to 71 months	53.6± .785
8 to 11 months	69.6±1.123	72 to 96 months	53.4± .669
12 to 15 months	67.8± .821		

[1] 20 determinations of blood sugar on not less than eight animals were averaged for each age interval.
[2] gm and cm³ are the abbreviations recently adopted by the Government Printing Office for milligrams and cubic centimeters, respectively.

It is apparent that during the early stages of life there is a close inverse relationship between the blood-sugar content and the age of dairy cattle. A mean of 100.4 ± 1.685 milligrams sugar per 100 cubic centimeters of blood was obtained in this study for calves less than 1 week of age. As the animal grows older its blood-sugar concentration decreases until it averages approximately 54 milligrams, when the animal is 2 years old. After this age is reached, the blood-sugar level does not seem to be influenced to any appreciable degree by an increase in age.

Two hundred and twenty-two observations made during the winter months on 74 animals between 2 and 8 years of age gave a mean blood-sugar concentration of 53.03 ± 0.297 milligrams per 100 cubic centimeters of blood, with values ranging from 35 to 74 milligrams.

BREED

The mature cows in the herd were used for the study of the influence of breed on blood-sugar content. By using only mature aninals it was sought to exclude the age factor. Stage of lactation was not considered since these studies have shown it to have little influence on the blood-sugar concentration.

No significant difference was observed in the blood-sugar content of the various breeds. An average of 44 determinations on each of the four major breeds gave the following means: Ayrshire, 53.1 ± 0.856; Guernsey, 53.6 ± 0.711; Holstein, 52.8 ± 0.440; and Jersey, 52.5 ± 0.459.

MILK YIELD

Hewitt (7) has recently reported results with dry and lactating cows. Eight determinations on three dry cows gave an average of 90.4 milligrams blood sugar, while a similar number on three lactating cows gave an average of 50.7 milligrams. From this he concluded that the blood-sugar level in dry cows is decidedly higher than for cows in milk. Theoretically, the withdrawal of sugar from the blood

to form the lactose of milk might lower the blood-sugar level in lactating cows. It does not seem probable, however, that this factor alone would explain the wide difference observed.

In order to study the influence of milk yield on blood-sugar level, all the available data in these studies were grouped according to daily milk yield, as shown in Table 2. The results on heifers were not grouped with those on dry cows, since, as already shown, young animals have a higher blood-sugar level than mature animals. The difference in the means of the dry and the heaviest producing group (1.9 ± 1.16) is less than twice its probable error, which is not generally regarded as significant. However, a small difference is apparent in the figures presented, and a slight negative correlation (0.190 ± 0.038) was obtained in correlating blood sugar and milk production for the three producing groups in Table 2.

TABLE 2.—*Influence of milk yield on blood-sugar level in dairy cattle*

Daily milk yield	Determinations	Cows	Mean value for blood sugar
	Number	*Number*	*Mg per 100 cm³*
Dry	42	20	52.6±0.843
1 to 15 pounds	27	21	52.9± .455
16 to 35 pounds	64	33	51.6± .514
Over 35 pounds	32	15	50.7± .799

While these results are lower than those reported by Schlotthauer (*13*), in general, they confirm his finding that heavy producing cows have a slightly lower blood-sugar level.

FEEDING

It is a well-recognized fact that the blood-sugar level may be influenced by the rate of absorption from the intestines. In animals in which the process of digestion is rapid and more or less intermittent, the blood-sugar level varies considerably throughout the day. After a meal of readily digested carbohydrates it increases for a time, but returns to a lower level as the rate of absorption decreases. To observe whether or not such a fluctuation occurred in dairy cows fed in the normal way, the blood sugar was determined at intervals during the day. The cows were fed at 6 a. m. and 4 p. m. Blood samples were taken at 7 and 10 a. m. and at 1, 3, and 5 p. m.

TABLE 3.—*Variation in blood-sugar content in 22 dairy cattle at intervals throughout the day*

Hour of day	Time elapsed since feeding	Mean value for blood sugar
	Hours	*Mgm. per 100 c. c.*
7 a. m.	1	51.4±0.834
10 a. m.	4	49.0± .655
1 p. m.	7	50.7± .733
3 p. m.	9	52.9± .475
5 p. m.	1	50.5± .882

The results, which are summarized in Table 3, indicate that no significant difference in the blood-sugar level of the dairy cow is produced by feeding. This is undoubtedly due to the rather slow and continuous process of digestion resulting from the complex nature of the bovine stomach.

It will be observed from Table 3 that there is a small increase at 3 p. m., which probably can be accounted for by the increased activities about the barn at this time in preparation for the afternoon feeding.

That the rate of absorption of sugar from the digestive tract does influence the blood-sugar level in dairy cows is indicated by the results of the two following experiments—the first on the influence of fasting, and the second on the sugar tolerance of the dairy cow.

FASTING

Five dairy heifers were used in studying the influence of inanition on the blood-sugar content. These heifers ranged in age from 1½ to 2 years and comprised 2 Holsteins, 1 Ayrshire, 1 Guernsey, and 1 Jersey. Throughout the trial they were kept in a paddock with shed adjoining. Water was available at all times.

Samples of blood for analysis were drawn daily, beginning two days before the fasting period, and the live weights of the animals determined. At the conclusion of the 9-day fasting period the heifers were gradually returned to normal feeding conditions.

Figure 1 shows the results in graphic form. It will be observed that as the fasting period advanced the concentration of the blood sugar decreased. This decrease was continuous and uniform until the morning of the seventh day, when a substantial increase occurred. This increase may be explained by the fact that the heifers broke through the fence and obtained some roughage the evening before. As the fasting continued the blood sugar decreased more than 50 per cent of its initial content. The lowest average value observed was 28.5 milligrams per 100 cubic centimeters blood for the eighth day of the trial, as compared with an average initial content of 61.2 milligrams.

After nine days without feed the heifers appeared gaunt and inactive. Their feces were watery and contained mucous material, though it is unlikely that all of the contents had been removed from the digestive tract in the short time involved.

The blood-sugar content of the heifers did not return to normal immediately after they were fed, but increased rather slowly for several days. The average live weight of these heifers decreased 120 pounds.

ADMINISTRATION OF GLUCOSE

In order to secure more rapid absorption of carbohydrate from the digestive tract, trials were run in which sugar was dissolved and given by means of a stomach pump. The animals in the various tests were fed different amounts of glucose according to their size and capacity. The glucose used was dissolved in water at body temperature and introduced slowly by means of a stomach pump. That much of the solution found its way immediately into the abomasum and was readily available for absorption is indicated by the prompt rise in blood sugar that occurred in a majority of the animals under observation.

The solutions were administered at 9 a. m., immediately after an initial blood sample had been taken. Samples were then secured at 30 minutes, 1, 2, 4, and 7 hours after dosage. In the first two trials the animals had been deprived of feed for 12 hours. In the remainder

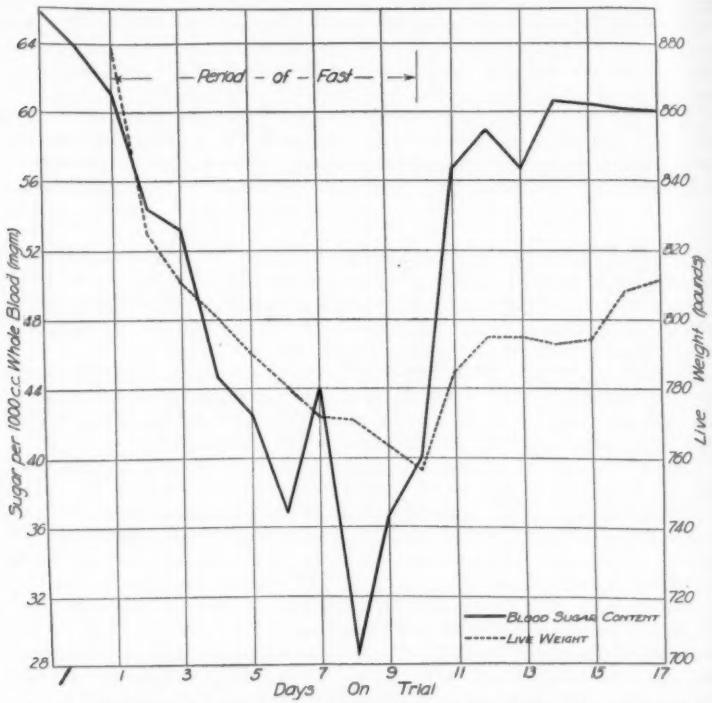

FIGURE 1.—Influence of fasting on the blood-sugar level and live weight of dairy cattle. The sudden rise in blood sugar on the seventh day was undoubtedly caused by the fact that the heifers broke through the fence and obtained some roughage the evening before

no attempt was made to withhold any part of the regular ration prior to giving the sugar.

TABLE 4.—*Effect of the administration of glucose on the blood-sugar level of dairy cows*

Breed and daily milk record of animal	Glucose administered	Milligrams sugar per 100 cubic centimeters whole blood at time indicated					
		Before dosage	30 minutes after dosage	1 hour after dosage	2 hours after dosage	4 hours after dosage	7 hours after dosage
	Pounds						
Holstein, dry	8	43.1	108.7	133.3	156.2	114.9	54.0
Holstein, dry	6	47.8	78.7	69.0	105.3	91.0	59.5
Guernsey, 9 pounds	7	67.6	84.7	94.3	133.3	64.5	52.6
Guernsey, 10 pounds	7	50.0	84.0	113.6	166.7	52.4	50.0
Holstein, 40 pounds	8	59.2	137.0	166.7	88.5	83.3	63.3
Guernsey, 25 pounds	6	54.0	117.6	105.3	100.0	76.9	58.8
Guernsey, 24 pounds	8	50.0	77.5	100.0	90.0	71.9	57.1

The results (Table 4) show a marked increase in blood-sugar concentration after dosage. This increase reached a maximum within approximately 2 hours, and 6 or 7 hours were required for the concentration to return to normal. In some of these trials it was possible to increase the blood-sugar content more than threefold.

That all this increase was not due to excitement resulting from the use of the stomach tube was shown by a repetition of the experiment in which water was used in place of the sugar solution. The average determination on four animals before the water was introduced was 65.3 milligrams of blood sugar. Thirty minutes after dosing the quantity had increased to 69.5 milligrams; one hour later it had decreased to 62.2 milligrams.

In every case the qualitative test (*8*) for sugar in the urine gave negative results for urine samples collected prior to the administration of glucose. Except in one trial, in which the blood sugar did not materially increase, sugar appeared in the urine within two to four hours after the solution was given. This glycosuria, which was to be expected under the conditions of the experiment, indicates that the "sugar threshold" value of the kidneys had been temporarily exceeded.

It is evident from these results that when soluble carbohydrate is given in such a way as to permit rapid absorption from the intestinal tract, a marked increase in the level of blood sugar results. This method of increasing the blood-sugar level has been used by the writers in studying the factors influencing lactose formation by the dairy cow.

EXCITEMENT

In order to determine the effect of excitement, the blood sugar of four cows was determined before and after a dog was brought into the barn. The nervousness of the cows resulted in an average increase in the blood sugar, ranging from 58.5 to 65.9 milligrams. When the dog was allowed to bark the blood sugar was further increased to 89.1 milligrams. All four cows showed a definite increase in this constituent.

Any undue excitement of an animal while the sample of blood is being taken for analysis may produce a marked increase in the blood sugar. This is particularly true when the animal is bled from the jugular vein. Unless one has had considerable practice in securing the blood in this manner, the animal may be rendered extremely nervous before the sample is finally obtained. In the work reported in this paper, the method used required only 0.1 cubic centimeter of blood, which could be taken quite conveniently from the ear, with a minimum of discomfort to the animal. The uniformity of the results recorded in Tables 1 to 4 may probably be attributed to this and the uniform conditions under which the samples were taken.

OESTRUS

A limited number of observations were made on cows during oestrus. In each case studied there was a distinct rise in the blood sugar, amounting in two instances to as much as 40 per cent. Hewitt (*7*) has reported blood-sugar values as high as 362 milligrams for heifers during oestrus. Observations made on five heifers in this herd gave no value in excess of 90 milligrams.

TEMPERATURE

As already stated, the results recorded in these studies were secured during the winter months, when the cows stayed in the barn a large part of the time and were maintained under uniform conditions. Some determinations made since in another study during extremely warm July and August weather gave results considerably higher than any observed heretofore. Further investigation will be required to determine definitely whether the increase in the blood-sugar level was due to the high temperature prevailing at the time the tests were made or to other factors.

SUMMARY

In the course of this investigation, blood samples from 140 dairy cattle were analyzed for sugar content. The following results were obtained.

Calves shortly after birth had a blood-sugar content of about 100 milligrams per 100 cubic centimeters. As the age of the animal increased the blood sugar decreased until the animal was approximately 2 years of age, after which little further change was observed. A mean blood-sugar content of 53.03 ± 0.297 milligrams per 100 cubic centimeters of blood was obtained on 222 samples from 74 animals between 2 and 8 years of age, with a range of 35 to 74 milligrams.

No significant difference was observed in the blood-sugar level of the four breeds studied.

Cows giving a liberal flow of milk were found to have slightly less blood sugar than dry cows or those yielding a small quantity of milk.

There was no increase in blood sugar after feeding. A slight increase was observed at 3 p. m., which probably may be accounted for by the increased activities about the barn prior to the afternoon feeding.

Fasting caused a decrease in the blood-sugar content of dairy heifers amounting approximately to 50 per cent.

The administration of glucose in solution produced increases in the blood-sugar content amounting to as much as 200 per cent.

Excitement was found to produce a marked increase in blood sugar.

The blood-sugar values of cows and heifers were higher during oestrus than at other times.

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THE GERMINATION OF COTTONSEED AT LOW TEMPERATURES [1]

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INTRODUCTION

One of the most important factors in the production of a large crop of cotton is a full stand of plants early in the season. Studies concerning the effect of date of planting on yield carried on at the South Carolina Experiment Station for the past six or seven years have shown that the cotton planted earliest produces the highest yield if a permanent good stand is secured. But the period of greatest mortality of cotton plants due to adverse environmental conditions is from the time the seed is planted until the seedling stage is past. It is during this time that cold wet weather so often makes early planted cotton a failure. It follows, therefore, that if a variety of cotton, excellent in other respects, could be found with more resistance to cold in the seedling stage, farmers would be able to plant earlier or would be assured of better stands from plantings at the usual time. In either case they would secure greater yields.

The isolation or development of such a variety by means of field selections is not easy. One season may be suitable for such selections and the next one totally unsuitable. It is therefore very desirable to devise a better method for making the selections, if it can be done. A possible method of doing this was suggested by results secured with corn by B. D. Leith,[3] of the Wisconsin Agricultural Experiment Station. By a method which consisted in part of testing the seed in a refrigerator and selecting for planting only those ears from which germinations occurred within a given time, he was able to produce a hardier strain of corn than the earliest then grown in Wisconsin. As part of an attempt to adapt this method to the selection of cold-resistant strains of cotton, a study of the germination behavior of the seed of a number of varieties was made. The chief results are reported in this paper.

METHODS

Briefly stated, the method used in these experiments was to germinate samples of the best seed of different lots at approximately the minimum temperature at which germination would occur, and then to compare the behavior of the different strains and individuals. The percentage germination was not of itself considered especially

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[2] The writer is indebted to Dr. C. F. Hottes and to the authorities of the University of Illinois for the privilege of using the constant-temperature chambers in the laboratories of plant physiology of that institution during the first months of 1926; to the late F. T. Dargan, professor of electrical engineering at Clemson Agricultural College, for much of the design and construction of the low-temperature incubator used in the later work; to T. L. W. Bailey, jr., and C. C. Bennett, who assisted with some of the germinations; and to the large number of others who generously contributed samples of cottonseed for the work.

[3] RUSSELL, H. L., MORRISON, F. B., and EBLING, W. H. COLD RESISTANT CORN FURTHER DEVELOPED. Agr. Expt. Sta. Bull. 373 (Ann. Rpt. 1923–24); 25–26, illus.

significant. The information desired was what the viable seed would do at the low temperature. For this reason all apparently defective or damaged seeds (lightweight, pale, gin cut, etc.) were discarded, and parallel tests of all samples at 25° to 30° C. were run. The higher temperature was assumed to be near the optimum; and, while it was not definitely so proved, the promptness and high percentage of germination repeatedly secured indicated that the assumption was not far wrong. It is clear, for instance, that a variety germinating 50 per cent at the low temperature would be distinctly superior from the standpoint of resistance to cold to one germinating 75 per cent, if the respective germination rates at the optimum should be 50 and 95 per cent, respectively. In the first case 100 per cent of the viable seed would germinate at the low temperature while in the latter only 79 per cent would do so. The germination percentages reported at the low temperatures are therefore based on the number of seeds which the test at the high temperature showed to be viable.

The germinations reported are for time intervals commencing with the beginning of the test and ending with the close of the week mentioned. Since in a study of this kind the number of seeds which germinate, say, by the end of the second week, is more important than the number which germinate during the second week of the period, the germination percentages by individual weeks are not given. The values are not always given in detail after the fourth week, although the experiments were often conducted longer.

Preliminary experiments to determine the most suitable method of germinating the seed included germination on plaster of Paris blocks in water and in wet sand, both with and without a cloth or paper covering; germination in a shallow layer of water; and germination between moist absorbent papers. The last method was the most satisfactory; and the best plan found for keeping the papers properly moist was to lay the lower one on a uniform layer of sand about one-half inch deep saturated with water in a covered, ventilated tray or pan. The suitability of delinting the seed with sulphuric acid as compared with the prewetting method of Toole and Drummond [4] was also investigated. The delinted seeds, as shown in the first part of Table 1, germinated better, partly no doubt because delinting made it possible to detect and remove most of the defective seeds. Delinting was therefore adopted as the method of preparing the seeds for the tests.

Examinations were made daily so far as possible. Temperatures were taken by means of thermometers lying on the upper layer of paper, germinated seeds were removed and counted, and water was added to replace that lost by evaporation. In the earlier part of the work it was found difficult after opening a tray to get the thermometer reading before the warmer air of the room had caused it to change. Later this difficulty was overcome by tying some filter paper around the bulb. With this treatment there was abundant time for noting the reading before any change occurred.

It should be noted that the actual effective temperature was really somewhat higher than that observed, even when the readings were most accurately taken. This was owing to the fact that tem-

[4] TOOLE, E. H., and DRUMMOND, P. H. THE GERMINATION OF COTTONSEED. Jour. Agr. Research 28: 285-291, illus. 1924.

perature readings were made immediately after the trays were removed from the incubator for examination. During the course of the examination the temperature inevitably rose somewhat, since the trays were necessarily exposed to the warmer air of the room during that time. There were also a few occasions when the refrigerating system failed to operate properly. During the first year in particular this happened more often in the later, warmer part of the season than earlier. For this reason, and because the air to which the seeds were exposed during observations was warmer in the later part of the season, the effective temperature under which germination occurred was higher each season when the last lots were germinated than when the first were germinated. In order to get a measure of the effect of these environmental variations, a strain of Cleveland (lot No. 1, Tables 1 and 2) was selected as a standard and a sample was included with every group of samples germinated. It proved to be a very high grade lot of seed from the standpoint of germination at the higher temperatures. Some of the samples gave perfect germination and none failed to give a very high one.

Enough water was sprinkled on the covering paper after the daily examination had been made to make it appear as moist as at the beginning of the experiment. Tests were conducted to determine whether the inaccuracies of this method of replacing evaporated water were such as to introduce significant variations in the percentage of germination. The results showed that too much or too little water would decrease germination, but the variations in water content involved in these tests were large enough to be easily detectable by appearance. As a matter of fact, the paper very quickly took on a slightly dry appearance if the level of free water fell below the surface of the sand, and became soaked with water if the level rose above the surface. There seems to be no reason to suspect that better germinations could have been secured with a different amount of water in the sand or that the variations in moisture were sufficient to influence the results appreciably.

After samples had been kept at the low temperature until no more germinations occurred or until other circumstances made it necessary to discontinue the test, they were placed at the high temperature, 25° to 30° C., for a time to determine how many, if any, of the remaining seeds were still alive.

The earliest germinations were carried out in a small, improvised incubator composed of a wooden box set in an ice-cooled refrigerator and equipped with electric heating units and a very simple thermostat. The work in the early months of 1926 was done in the laboratories of plant physiology at the University of Illinois. The constant-temperature chambers built and operated by C. F. Hottes[5] were used in these tests. The last two seasons' germinations were made at Clemson College in a constant-temperature chamber built for the purpose. It consisted of an insulated box placed in an electric refrigerator and equipped with heating coils, thermostat, and air stirrer. With this apparatus it was possible to get satisfactory

[5] HOTTES, C. F. A CONSTANT HUMIDITY CASE. (Abstract) Phytopathology 11: 51. 1921.
——— COOPERATIVE RESEARCH IN PLANT PHYSIOLOGY AND AGRONOMY. Jour. Amer. Soc. Agron. 18: 60-68, illus. 1926.

control and practical uniformity of the temperature. Thermometer readings indicate that there may have been at times a maximum of 0.3° C. difference in temperature between the bottom tray and the top one. In order to equalize the effect of any such difference the position of the trays in the incubator was changed regularly.

RESULTS

GERMINATION BEHAVIOR AT LOW TEMPERATURES

Preliminary experiments made with extemporized equipment in 1925 with a few varieties of upland cotton indicated that the minimum temperature for germination is approximately 12° C. The results of the later experiments are shown in detail in Tables 1 and 2. While not permitting the precise determination of the minimum temperature for germination, they fully confirm the original results in a general way. They indicate that cottonseed might possibly germinate at slightly lower temperatures if given enough time, but that it is far from likely that it would germinate at an appreciably lower temperature than 11°, especially since, as already explained, the actual effective temperature in the tests was slightly above that recorded. Certainly the germinations would not proceed at a lower temperature at a rate that would make such tests practical for selection work.

TABLE 1.—*Comparative germination of cottonseed at 12°, 15°, and 25°–30° C.*

Lot No.	Variety	Source	Treatment	Date of start	Germination at 12°							Germination at 15°							Germination at 25°–30°	
					Seeds used	Seeds germinated after—				Total germinated	Germinated later at 25°	Seeds killed	Seeds used	Seeds germinated after—				Total germinated	Germinated later at 25°	Seeds killed	Seeds used	Total germinated
						1 week	2 weeks	3 weeks	4 weeks					1 week	2 weeks	3 weeks	4 weeks					
				1926	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*No.*	*Pct.*
2	Delfos 631	Delta Experiment Station, Stoneville, Miss.	Delinted	Feb. 19	100	-----	1.1	9.8	26.1	28.3	1.9	70.7	100	35.9	88.1	89.1	90.2	90.2	0	9.8	100	92.0
			Prewetted	do	100	-----		10.3	20.7	23.0	0	77.0	99	26.7	74.3	81.3	-----	81.3	0	18.7	100	87.0
3	Dixie Triumph	L. U. Watson Seed Co., Florence, S. C.	Delinted	do	100	-----	-----	2.1	7.4	27.4	10.5	62.1	97	27.1	69.5	76.0	-----	77.0	0	23.0	100	95.0
			Prewetted	do	100	-----	-----	1.2	2.4	11.9	3.6	84.5	102	17.5	63.0	73.5	77.0	77.0	0	23.0	100	84.0
4	Lightning Express No. 4	Florida Agricultural Experiment Station, Gainesville, Fla.	Delinted	do	100	-----	-----	2.1	5.2	36.5	27.1	36.5	100	51.0	93.8	97.9	-----	99.0	0	1.0	100	96.0
			Prewetted	do	99	-----	-----	-----	4.3	17.2	16.1	66.7	100	44.7	79.8	84.0	87.2	87.2	0	12.8	100	94.0
5	Rowden	E. A. Price, Garland, Ark.	Delinted	Feb. 20	100	-----	2.5	[a]3.8	-----	3.8	0	96.3	103	47.3	59.5	[a]60.7	-----	60.7	0	35.6	100	80.0
			Prewetted	do	100	-----	8.1	[a]9.5	-----	9.5	0	90.5	99	65.5	75.1	[a]79.2	-----	79.2	0	20.8	100	74.0
6	Acala	John D. Rogers, Navasota, Tex.	Delinted	Feb. 19	100	-----	-----	-----	3.3	17.6	25.3	57.1	99	44.0	82.4	85.7	92.3	92.3	2.2	4.5	100	91.0
			Prewetted	do	100	-----	-----	-----	2.3	2.3	0	97.7	99	21.8	41.4	50.7	57.5	57.5	0	41.9	100	87.0
1	Cleveland	Piedmont Pedigreed Seed Farm, Commerce, Ga.	Delinted	Feb. 17	100	-----	-----	1.1	2.3	8.0	56.0	36.0	102	3.9	31.3	65.7	69.6	75.5	7.0	17.6	100	100.0
			Prewetted	do	100	-----	-----	1.0	1.0	4.4	23.3	72.3	100	-----	17.3	27.4	36.1	46.2	4.3	49.5	101	69.3
7	Trice	Kentucky Agricultural Experiment Station, Lexington, Ky.	Delinted	Feb. 18	99	-----	-----	-----	-----	1.0	64.7	34.4	99	2.1	12.5	40.7	69.9	81.3	4.2	14.6	98	96.8
			Prewetted	do	99	-----	-----	-----	1.0	1.0	8.2	84.9	99	-----	5.5	16.6	29.1	31.8	1.4	66.9	100	73.0
8	Delfos	do	Delinted	Feb. 19	100	-----	-----	-----	-----	3.1	39.2	57.7	100	9.3	29.9	39.2	54.6	66.0	18.6	15.5	100	97.0
			Prewetted	do	100	-----	-----	-----	-----	2.2	6.5	91.3	100	10.9	34.8	34.8	43.5	50.0	8.7	41.3	100	46.0
9	Acala	do	Delinted	do	100	-----	-----	-----	-----	3.0	27.3	69.7	101	16.0	36.0	60.0	87.0	94.0	4.0	2.0	100	99.0
			Prewetted	do	100	-----	-----	-----	-----	3.2	1.1	95.7	100	15.1	31.2	45.2	68.8	69.9	2.1	28.0	100	99.0
10	Cleveland	do	Delinted	Feb. 18	100	-----	-----	-----	-----	3.0	61.7	35.4	100	1.0	16.2	47.5	68.7	81.8	3.0	15.2	100	93.0
			Prewetted	do	102	-----	-----	-----	1.4	13.6	13.6	72.7	94	-----	7.4	22.2	32.5	35.5	0	64.5	100	72.0
11	Express	do	Delinted	do	99	-----	-----	-----	-----	2.1	55.7	42.3	100	14.3	35.7	42.9	60.2	79.6	13.4	7.1	100	98.0
			Prewetted	do	100	-----	-----	-----	-----	0	11.7	88.3	99	10.8	22.6	35.5	48.4	55.9	4.3	39.9	100	94.0
12	King	do	Delinted	do	100	-----	-----	-----	4.7	4.7	0	95.3	101	-----	2.1	7.3	15.6	30.2	12.5	59.3	100	94.0
			Prewetted	do	100	-----	-----	-----	-----	0	30.8	69.1	102	-----	-----	4.6	9.2	13.8	4.6	81.6	100	43.0
13	College No. 1	Georgia State Agricultural College, Athens, Ga.	Delinted	Mar. 16	200	-----	23.6	61.5	64.9	71.8	1.7	26.4	200	36.2	77.6	82.2	[b]83.3	83.3	1.1	13.8	200	87.0
14	Cook 588–219	E. F. Cauthen, Auburn, Ala.	do	do	200	-----	15.0	46.0	56.2	65.2	18.7	16.0	200	22.5	62.0	73.8	[b]76.5	76.5	1.6	21.9	200	93.5

[a] Fungal infections so severe that 12° and 15° lots were discarded in the middle of the third week.

TABLE 1.—*Comparative germination of cottonseed at 12°, 15°, and 25°–30° C.*—Continued

Lot No	Variety	Source	Treatment	Date of start	Germination at 12°							Germination at 15°							Germination at 25°–30°	
					Seeds used	Seeds germinated after—				Total germinated	Germinated later at 25°	Seeds killed	Seeds used	Seeds germinated after—				Total germinated	Germinated later at 25°	Seeds killed	Seeds used	Total germinated
						1 week	2 weeks	3 weeks	4 weeks					1 week	2 weeks	3 weeks	4 weeks					
				1926	No.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	No.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	No.	Pct.
15	Delta and Pine Land No. 4.	Delta & Pine Land Co. of Mississippi, Scott, Miss.	Delinted	Mar. 16	200	.....	9.7	41.0	52.3	60.0	19.5	20.5	200	7.2	53.9	79.0	[a]87.7	87.7	4.1	8.2	200	97.5
16	Snow Flake	John McLernon, Clarksville, Tex.	do	do	200	.....	7.6	43.7	52.8	59.9	11.7	28.4	195	2.1	75.5	84.9	[b]90.1	90.1	4.2	5.8	200	98.5
17	Covington Toole	Florida Agricultural Experimental Station.	do	do	200	.....	7.5	53.5	62.0	69.0	5.9	25.1	200	6.4	87.7	92.0	(b)	92.0	2.1	5.9	200	93.5
18	New Boykin (stock No. 852).	Ferguson Seed Farms, Sherman, Tex.	do	do	200	.....	5.8	22.6	37.4	48.4	36.9	14.7	200	3.7	67.9	86.8	[b]91.6	91.6	3.7	4.7	200	95.0
19	Mebane	A. D. Mebane Sales Agency, Lockhart, Tex.	do	Mar. 15	200	.....	4.3	32.8	35.5	35.5	.5	64.0	200	.....	48.4	64.5	.....	64.5	.5	34.9	200	93.0
20	Startex No. 333 (s selection from Lone Star).	Texas Agricultural Experiment Station, College Station, Tex.	do	do	200	.....	3.9	35.6	46.1	48.3	16.7	50.0	200	.....	72.2	81.1	82.2	82.2	0	17.8	200	90.0
21	Name unknown	(Donor not known), Rocky Mount, N. C.	do	Mar. 16	200	.....	1.6	28.6	45.3	55.2	20.3	24.5	200	2.6	73.4	88.0	[b]93.2	93.2	1.0	5.7	200	96.0
22	Carolina Foster	Humphrey-Coker Seed Co., Hartsville, S. C.	do	do	200	.....	1.6	11.8	19.3	20.9	8.6	70.6	200	1.1	24.6	46.0	[b]47.6	47.6	2.1	50.3	200	93.5
23	Super Seven	Pedigreed Seed Co., Hartsville, S. C.	do	Mar. 15	200	.....	.....	33.2	43.4	56.6	18.9	29.6	200	.....	40.3	77.0	83.7	83.7	6.6	9.7	200	98.0
1	Cleveland	Piedmont Pedigreed Seed Farm, Commerce, Ga.	do	do	201	.....	.....	6.5	16.4	25.4	45.8	28.8	200	.....	4.5	34.0	56.5	56.5	29.0	14.5	200	100.0
24	Pima	Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.; grown in Arizona.	do	Apr. 1	200	38.8	[c]80.6	90.3	93.4	93.4	0	6.6	200	96.4	[d]98.5	.....	.....	98.5	0	1.5	200	98.0
25	Sea island	Bureau of Plant Industry; grown near Charleston, S. C.	do	do	200	.....	[c]36.1	39.1	.....	39.1	0	60.9	200	33.8	[d]52.6	54.1	.....	54.1	0	45.9	200	66.5
26	Rowden	Bureau of Plant Industry; grown at Wills' Point, Tex.	do	do	200	.5	[c]11.4	50.0	62.5	62.5	3.3	34.2	200	56.5	[d]84.2	87.5	88.0	88.0	0	12.0	200	92.0
27	Kekchi	Bureau of Plant Industry; grown at Clarksville, Tex.	do	do	200	1.0	[c]6.3	42.9	79.6	79.6	15.2	5.2	200	26.7	[d]51.3	82.2	91.1	91.1	2.6	6.3	200	95.5

28	Belton, T. S. No. 5984–91	Texas Agricultural Experiment Station (substation No. 5), Temple, Tex.	do	Apr. 9	100	1.0	6.2	71.1	[e]74.2	74.2	11.3	14.4	200	21.1	89.7	91.2		91.2	1.5	7.2	200	97.0
29	Lankhart	Lankhart Bred Seed Farms, Waco, Tex.	do	do	200		4.8	15.1	[e]15.9	15.9	2.4	81.7	200	.8	31.7	[f]37.3		37.3	2.4	60.3	200	63.0
30	Wacona	do	do	do	100		4.4	21.0	[e]24.3	24.3	15.5	60.3	150	2.2	58.1	65.4		65.4	1.5	33.0	191	90.6
31	Kasch	Bureau of Plant Industry; grown at San Marcos, Tex.	do	do	200		1.6	66.1	[e]66.7	66.7	4.7	28.6	200	2.6	76.0	[f]84.4		84.4	2.1	13.5	200	96.0
32	Lone Star	Bureau of Plant Industry; grown at Greenville, Tex.	do	do	200		1.0	46.2	[e]48.2	48.2	29.2	22.6	200	.5	32.3	[f]49.2		49.2	25.1	25.6	200	97.5
33	Sunshine	J. W. Davidson, McKenney, Tex.	do	do	200		.5	66.3	[e]68.9	68.9	14.8	16.3	200	1.5	79.1	[f]84.2		84.2	9.7	6.1	200	98.0
1	Cleveland	Piedmont Pedigreed Seed Farm.	do	do	200			17.9	[e]20.4	20.4	[e]75.5	4.1	200		40.3	62.2	62.8	62.8	[e]36.7	.5	200	98.0
[h]34	Gossypium Nanking, Manchurian Black Seed Kuro-tane, C. B. 477.	South Manchuria	do	do	35		42.9	85.7	(e)	85.7	11.4	14.3	35	42.9	97.1			97.1	2.9	0	35	100.0
[h]35	G. Nanking, Manchurian White Seed, Shiro-tane, C. B. No. 478.	do	do	do	35		41.2	91.2	(e)	91.2	2.9	5.9	35	44.1	88.2	91.2		91.2	0	8.8	35	97.1
					No.		*No.*	*No.*	*No.*	*No.*	*No.*			*No.*	*No.*	*No.*	*No.*	*No.*	*No.*			*No.*
[h]36	C. B. 292	Villarica, Paraguay	do	do	10			5	(e)	5	1		10	2	3	4		4	2			
[h]37	Tree cotton, C. B. No. 286–A.	Mexico	do	do	10			1	(e)	1	0		10					0	0		10	1
[h]38	Peruvian tree cotton, C. B. 246–C.	Guatemala	do	do	10				(e)	1	5		10	1	7	8		9	1		(g)	
[h]39	Caravonica, C. B. No. 243.	Guatemala City	do	do	10		2	3	(e)	3	0		10	2				2	1		(g)	
[h]40	Caravonica hybrid C. B. 127–E.	Baracoa, Cuba	do	do	10			1	(e)	1	5		10		2	3		3	3		(g)	
[h]41	San Lucas cotton, C. B. No. 229.	Lanquin, Guatemala	do	do	10			1	(e)	0	4		10			1		1	4		(g)	
[h]42	Villarica (white), C. B. No. 204.	Paraguay	do	do	10			3	(e)	3	1		(g)								(g)	
[h]43	Villarica (brown), C. B. No. 204.	do	do	do	10			1	(e)	1	0		(g)								(g)	
[h]44	Florida cotton, C. B. No. 250.	Key West, Fla	do	do	10				(e)	0	1		(g)								(g)	
[h]45	C. B. No. 185–½ B	Abyssinia	do	do	5			2	(e)	2	0		(g)								(g)	
[h]46	Haitian cotton, cotton Violet, C. B. No. 244.	Haiti	do	do	2				(e)	0	0		(g)								(g)	

[b] The fourth week in these instances was only 6 days.
[c] The second week in these cases is 6 days, third week 8 days, and fourth week 6 days; the temperature reached approximately 15° on the eighth day.
[d] The second week in these cases is 6 days, third week 8 days, and fourth week 3 days.
[e] The fourth week is only 1 day; the chamber reached 16° on the thirteenth day and 20° on the sixteenth day.
[f] Transferred to 25° chamber at end of third week.
[g] Left 4 days only at 25°.
[h] From the Office of Acclimatization and Adaptation of Crop Plants, Bureau of Plant Industry. Grown at their acclimatization gardens at Torrey Pines in southern California. Numbers refer to their breeding numbers.
[i] No seeds put to germinate, owing to small size of sample available.

TABLE 2.—*Comparative germination of cottonseed at 12° and 23°–30° C.*

Lot No.	Variety	Source	Germination at 12°														Germination at 25°–30°	
			Date of start	Mean temperature	Seeds used	Seeds germinated after—										Germinated later at higher temperature	Seeds killed	Seeds used	Total germinated
						1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks				
			1927	°C.	No.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	No.	P. ct.
47	Pima, 1926 crop	Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.; grown in Arizona.	Feb. 26	11.9	300	2.5	76.5	82.7	84.1							0	15.9	300	92.3
24	Pima, 1925 crop	do	do	11.9	300		55.9	71.5	75.0	76.0						0.7	23.3	300	96.0
20	Startex, No. 333	Texas Agricultural Experiment Station, College Station, Tex.	Feb. 28	11.5	200		8.3	26.5	38.7	42.0						2.8	55.2	200	90.5
13	College No. 1, 1925 crop	Georgia State Agricultural College, Athens, Ga.	Feb. 26	11.9	300		5.1	27.6	45.5	51.8						4.3	44.0	300	85.7
48	College No. 1, 1926 crop	do	do	11.9	300	.4	1.6	5.8	13.6	26.0						26.4	47.7	300	86.0
19	Mebane	A. D. Mebane Sales Agency, Lockhart, Tex.	Feb. 28	12.0	200		1.0	17.2	22.2	23.2						3.5	73.2	200	99.0
25	Sea island	Bureau of Plant Industry; grown near Charleston, S. C.	Feb. 26	11.4	196		1.0	7.8								1.0	90.3	200	52.5
15	Delta and Pine Land No. 4	Delta & Pine Land Co. of Mississippi, Scott, Miss.	Feb. 28	12.0	200		.5	15.2	47.2	53.3						10.2	36.5	200	98.0
12	King	Kentucky Agricultural Experiment Station, Lexington, Ky.	do	11.5	200		.5	3.6	10.3	14.4						14.4	71.3	200	97.5
1	Cleveland	Piedmont Pedigreed Seed Farm, Commerce, Ga.	Feb. 26	11.4	200			16.2	31.3	40.1						21.4	38.5	200	96.0
22	Carolina Foster	Humphrey-Coker Seed Co., Hartsville, S. C.	Feb. 28	12.0	200			8.3	22.4	26.6						7.3	66.1	200	96.0
23	Super Seven	Pedigreed Seed Co., Hartsville, S. C.	do	11.9	200			7.8	30.3	40.4						13.1	46.5	200	99.0
27	Kekchi	Bureau of Plant Industry; grown at Clarksville, Tex.	do	11.9	100			3.2	21.5	29.0						11.9	59.1	100	93.0
6	Acala	John D. Rogers, Navasota, Tex.	do	11.9	100			3.1	9.4	13.5						20.9	65.6	100	96.0
11	Express	Kentucky Agricultural Experiment Station, Lexington, Ky.	do	11.5	200			1.5	12.4	18.6						46.9	34.5	200	97.0
10	Cleveland	do	Feb. 26	11.4	200			.5	24.2	30.5						33.2	36.3	200	95.0
9	Acala	do	Feb. 28	11.9	202			.5	4.6	9.3						38.7	52.0	200	96.0
2	Delfos 631	Delta Experiment Station, Stoneville, Mis	do	11.4	200				5.8	12.2						3.7	84.1	200	94.5
8	Delfos	Kentucky Agricultural Experiment Station, Lexington, Ky.	do	11.4	200				3.6	6.6						26.0	67.3	200	98.0

24	Pima, 1925 crop	Bureau of Plant Industry; grown in Arizona	Apr. 29	11.7	200	1.6	42.7	51.6	52.1								.5	47.4	(a)			
49	Cleveland, Nos. 1 a-3-14-5-3-3	Pedigreed Seed Co	do	11.3	200	1.0	42.1	69.0	76.6	79.2							.5	20.3	200	98.5		
47	Pima, 1926 crop	Bureau of Plant Industry; grown in Arizona	do	11.7	200	2.2	40.1	53.6	55.2								0	44.6	(a)			
50	Cook No. 10-10	A. S. Bains, Oneonta, Ala	do	11.5	200	4.5	39.5	70.5	79.5	81.5							4.5	14.0	200	100.0		
31	Kasch	Bureau of Plant Industry; grown at San Marcos, Tex.	do	11.1	200	.5	38.0	54.6	53.1								0	46.9	200	96.0		
51	Cleveland	Marett Farm & Seed Co., Westminster, S. C	do	11.3	200		30.8	63.6	73.2	77.8							3.5	18.7	200	99.0		
26	Rowden	Bureau of Plant Industry; grown at Will's Point, Tex.	do	12.0	200	.6	24.1	47.0	58.4	63.3							0	36.7	200	83.0		
33	Sunshine	J. W. Davidson, McKinney, Tex	do	12.0	200		20.8	46.9	58.9	63.0							2.6	34.4	200	96.0		
4	Lightning Express No. 4	Florida Agricultural Experiment Station, Gainesville, Fla.	do	11.3	200		19.8	49.5	67.7	71.9							2.6	25.5	200	96.0		
52	Express	G. A. Hale, Burdette Plantation, Burdette, Ark.	do	11.1	200		15.5	55.2	76.8	78.9							1.3	20.1	200	97.0		
53	Trice	do	do	11.3	200		13.8	25.1	34.9	37.4							1.5	61.0	200	97.5		
54	Express No. 6	Pedigreed Seed Co	do	11.3	200		13.1	40.8	61.8	66.5							5.2	28.3	200	95.5		
30	Wacona	Lankart Bred Seed Farms, Waco, Tex	do	11.5	200		13.1	17.5	19.4								0	80.6	200	80.0		
29	Lankart	do	do	12.0	200		11.4	20.7	23.6								0	76.4	200	70.0		
28	Belton, T. S. No. 5984-91	Texas Agricultural Experiment Station (substation No. 5), Temple, Tex.	do	11.1	200		9.7	30.6	58.7	61.7							2.6	35.7	200	98.0		
13	College No. 1, 1925 crop	Georgia State Agricultural College	do	11.3	200		8.8	30.9	48.5	49.6							0	50.4	(a)			
55	Sea Island	Bureau of Plant Industry; grown near Charleston, S. C.	do	11.7	200		8.8	9.5									.7	89.8	200	68.5		
48	College No. 1, 1926 crop	Georgia State Agricultural College	do	11.3	200		4.1	15.7	42.4	49.4							7.6	43.0	(a)			
1	Cleveland	Piedmont Pedigreed Seed Farm	do	11.3	200		1.6	20.2	47.1	55.4							11.4	33.2	200	96.5		
11	Express	Kentucky Agricultural Experiment Station	do	11.3	200			5.6	33.3	47.5							19.2	33.3	200	99.0		
56	Gossypium nanking, Manchurian Black Seed, Kurotane, C. B. No. 477.	South Carolina Agricultural Experiment Station, Clemson College, S. C.; grown in 1927.	Dec. 17	11.7	300	22.4	42.9	72.8	81.6	87.1	87.4	89.1	89.8	89.8	90.1		1.7	8.2	300	98.0		
1	Cleveland	Piedmont Pedigreed Seed Farm	do	11.7	200	17.0	25.0	42.0	69.8	76.0	79.6	83.2					0	16.8	195	55.9		
57	Wannamaker Cleveland	Ben Roper, Calhoun, S. C.; grown in 1927	do	11.7	200		4.3	14.1	34.4	55.3	63.9	71.9	74.3	76.8	78.6		5.5	15.8	199	81.4		
58	Delfos No. 631 (selfed seed grown from lot 2).	South Carolina Agricultural Experiment Station; grown in 1927	do	11.9	200		1.0	5.1	14.9	30.8	39.5	49.2	54.4	56.9	60.0		10.3	29.7	200	97.5		
59	Cleveland (selfed seed grown from lot 1).	do	do	11.7	200			3.2	10.8	43.7	55.1	63.3	69.6	72.3	75.3		10.8	13.9	200	79.0		
60	Cleveland	do	Dec. 16	11.6	200			1.5	29.3	61.1	71.2	79.8	83.3	84.8	86.4		7.1	6.6	200	99.0		
61	Startex No. 333 (selfed seed grown from lot 20).	do	do	11.7	200			1.5	9.5	25.1	38.2	47.7	52.3	53.8	55.8		22.1	22.1	200	99.5		
62	College No. 1 (Selfed seed grown from lot 13).	do	do	11.6	200			.5	11.6	27.8	34.9	53.0	58.6	61.1	63.6		32.3	4.0	200	99.0		

[a] Germination test made in February used as a control here.

A second point worth noting here is that near the minimum for germination a small difference in temperature makes a much greater difference in the behavior of the viable seeds than it does at a higher temperature. Thus the unweighted mean of the total germinations at 12° C. as given in Table 1, omitting the foreign cottons represented by only a few seeds each, is 30.7 per cent, while that at 15° is 71.7 per cent. Similarly, the means for the seeds killed by the treatment are 51.4 and 23.4. In other words, reducing the temperature from that used as a control (25°–30°) to 15° reduced germination only 28.3 per cent (100−71.7 per cent), while reducing it 3° further reduced it 41.0 per cent (71.7−30.7 per cent). Likewise reducing the temperature to 15° killed 23.4 per cent of the viable seeds while the 3 degrees additional reduction killed 28.0 per cent more. The relative speed of germination, while not so easily summarized in a few figures, is very similar, as can be seen from Table 2. Another consideration showing the great variation in response resulting from a small change in temperature in this range is the fact that it was found practically impossible to repeat experiments with conditions enough alike to get identical results within the range of natural variation expected between samples. Any temporary failure of the regulatory apparatus to function, allowing the temperatures to rise for a short time, or apparently even merely the variation in room temperature where the daily examinations were made was enough to produce a significantly different behavior in the germination. Thus, owing to the slighly higher effective temperature in the later part of the season during the first two years, as explained under Methods, the germination became more rapid as the season advanced. While these results do not prove the point, they suggest rather strongly that the temperature coefficient for cotton germination in this range is considerably greater than at higher temperatures.

VARIETAL VARIATIONS

Reference to Tables 1 and 2 show that the varieties studied fall into two distinct classes with regard to the rate and percentage germination of the seed at temperatures near 12° C. In one class the rate is distinctly greater than in the other. The more rapidly germinating class includes the American-Egyptian variety, Pima, and two varieties, Manchurian Black Seed and Manchurian White Seed, of the Asiatic cotton, *Gossypium nanking*. There can be no question about the inclusion of Pima and Manchurian Black Seed in this group, as they were tested at different times with results that were consistent throughout. There is more question with regard to Manchurian White Seed, since in this case the placing is made on the basis of the preformance of only 35 seeds. However, in the one test made with the variety its behavior was so similar to that of Manchurian Black Seed that there seems to be no reason why it should not be placed in the same group. Figure 1 shows the germination of Pima in 1926 as compared with three other varieties after about 10 days in the germinator and Figure 2 shows the germination of Manchurian Black Seed as compared with College No. 1 in January, 1928. In examining these illustrations it should be recalled that the seeds were removed as soon as they germinated, so that a blank space represents a germinated seed. It may be mentioned in this connection that the finding with regard to Pima is in line with the general experience of farmers in the Southwest. In

sections where both Pima and upland varieties are grown Pima is planted earlier.[6]

The second class of cotton varieties investigated includes all those not already mentioned, with the possible exception of some of the foreign varieties of which only a few seeds were available. In this group there is apparently a considerable variation between varieties, although it is practically impossible to give any of them more than an approximate place in an arrangement based on the performance in question. An attempt has been made in the tables to make such an approximate arrangement for each separate group studied. Since it was found impossible to maintain conditions from one germination test to another such that the germination of the check variety would remain constant, and since there were likewise a number of as yet unexplained variations in the relative germinations of identical lots of seed, no attempt has been made to classify the group as a whole. About the only statement that it seems safe to make is that the strain

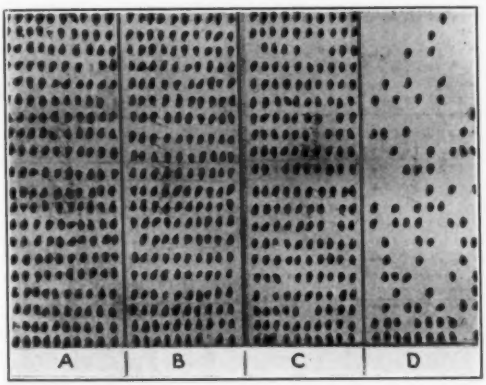

FIGURE 1.—Germination of seeds of Rowden (A), Kekchi (B), sea island (C), and Pima (D) cotton after about 10 days at 12° C. The samples originally contained 200 seeds each. Vacant places represent seeds which germinated and were removed. Test started April 1, 1926

of Cleveland used as a check stands well toward the foot. Cleveland is considered one of the earlier varieties; but its earliness, at least in so far as this strain is concerned, is apparently not due to ability to germinate and grow well in cool weather.

In this connection it is worth noting that failure to germinate at the low temperature does not necessarily mean that the seeds were killed. (Columns 12, 13, 20, and 21 of Table 1 and columns 17 and 18 of Table 2.) This particular lot of Cleveland seed, for instance, always had a large percentage of viable seed remaining after a month or more at each test until the last, at which time it was over 2 years old. Such a characteristic would often permit early planting, even if the weather were not at once suitable for germination. Whether this character is hereditary or merely a chance characteristic of the samples in which it was observed can not now be stated.

[6] Communication by C. B. Doyle, Bureau of Plant Industry, U. S. Department of Agriculture.

A possible explanation of some of the contradictory results mentioned above lies in the fact, to be discussed below, that some of the data suggest that the germination performance of cottonseed varies after a year or so. If this is true, the relative varietal performance might vary considerably from time to time, depending on the relative age at which seed of the different varieties undergoes the change. In this case, of course, it would be necessary to make varietal comparisons before the seed of any had undergone the change mentioned. Presumably almost any time within the first year after harvest would be safe.

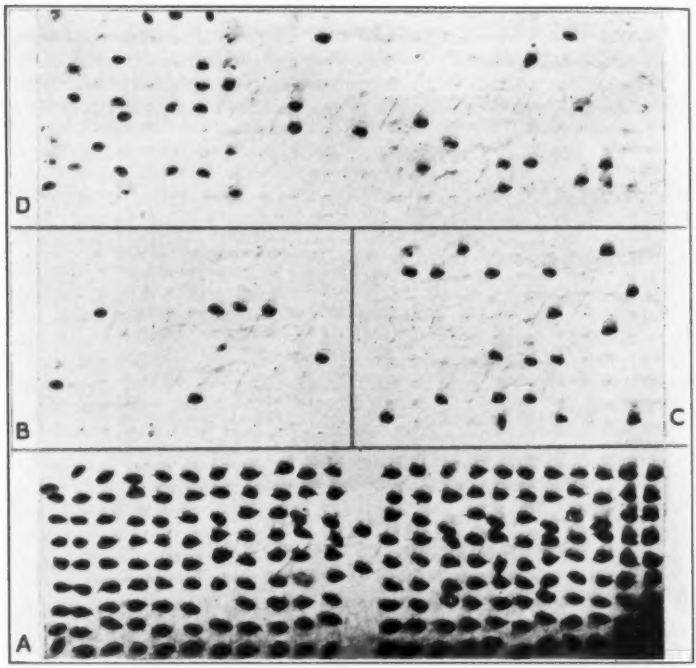

FIGURE 2.—Germination of seeds of College No. 1, lot 62 (A), *Gossypium nanking*, C. B. 477, lot 56 (C–D), and of a selection of the latter after 24 days at 12° C. Samples originally contained 200, 300, and 100 seeds, respectively. Seeds in (B) not considered in Table 2.) Seeds grown at Clemson College in 1927; test started December 16 and 17, 1927

It is probably true also that for a critical comparison of varieties where the difference is not very marked the seed should be grown in the same locality under as nearly identical conditions as possible and should be kept under identical storage conditions after picking. It is interesting to note in this connection that in all cases where Kentucky-grown seed was tested in comparison with seed of the same variety grown elsewhere (Tables 1 and 2) the seed grown elsewhere germinated more quickly at low temperature. But, owing to drought and extremely high temperature, the cotton from which the Kentucky-grown seed was taken opened prematurely. Thus, the indication

is clear that the seed was affected adversely by weather conditions while the crop was maturing. The effect was of such a nature as not to be detectable under conditions favorable for germination but easily detectable at low temperatures.

The considerations mentioned in the last two paragraphs may explain at least in part some of the contradictory results obtained with most of the upland varieties. However, there seems to be no reason for thinking them important enough to bring into question the finding concerning Pima, *Gossypium nanking*, or the strain of Cleveland mentioned. The performance of these strains was consistent throughout and extended through two generations in each case.

INDIVIDUAL VARIATIONS

Further reference to Tables 1 and 2 discloses the fact that there are among the seeds of most, and probably all, varieties a few that will germinate a week or more before the bulk of the germination occurs. Whether this earlier germination has a genetic basis or is only the expression of chance environmental conditions can only be learned by breeding trials, which in the present case have not gone far enough to determine this point. In the case of Pima and other pure-line varieties, the latter is more apt to be the case than with the more heterogeneous varieties. Although a pure-line variety may not have been selected for the character in question, still the repeated selfing employed in stabilizing it must have led to gametic purity in this regard as in others. However, the data indicate that variations occur often enough in the direction of increased ability to germinate at low temperatures to make comparatively easy the isolation by selection of strains notably strong in this regard, provided of course that such variations are in fact heritable. Whether the isolation of such strains can be considered worthwhile will depend, of course, on whether the ability in question is correlated with hardiness to cold in the seedling stage.

EFFECT OF AGE OF SEED ON GERMINATION IN THE COLD

As suggested above, age may affect the germination of cottonseed in the cold. It is very difficult to arrange experiments to test this hypothesis, owing to the great difficulty of maintaining exactly the same temperature conditions for the germination of successive lots of seed and to the relatively large effect which small temperature increments exert near the minimum. For this reason the comparative germination rates of successive samples of identical lots of seed can throw little light on the problem.

However, during the winter of 1926–27 old seeds of Pima and College No. 1 were twice tested in comparision with seeds of the same varieties of the succeeding season's crop. (Table 2.) With Pima the advantage in the first test was clearly with the younger seed, both as to rate of germination and number which germinated; in the second test the rates were practically the same. With College No. 1 a larger early germination was exhibited in both cases by the older seed, and in the first trial a greater total germination as well. In the second trial the total germination was about the same for the two lots.

In 1927–28 some 1925 seeds of Piedmont Cleveland were tested in comparison with selfed seeds grown locally in 1927 from the same lot. The older seed germinated more promptly. Figure 3 shows the two lots of seed on the twenty-fourth day after they were put to germinate.

The older sample, at the higher temperature, germinated more poorly than previously. What may perhaps be interpreted as confirmatory evidence is the fact, already mentioned, that the placement of varieties differed rather widely from test to test. This result would be expected if the seed of one variety passed through the change more rapidly than that of another.

On the whole, while the data can not be claimed to prove that an improvement in the ability of cottonseed to germinate in the cold

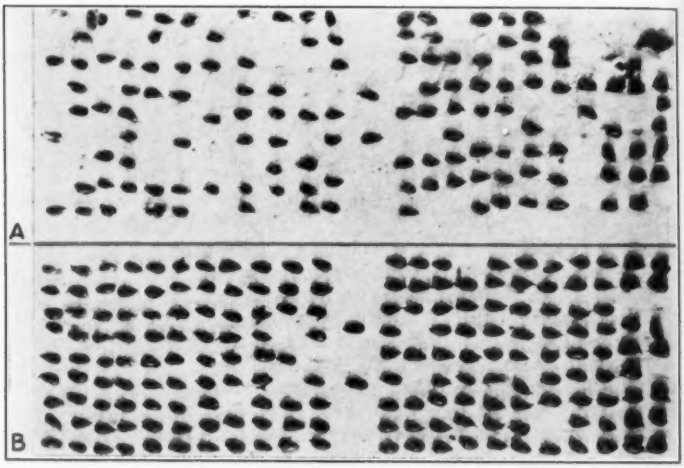

FIGURE 3.—Germination of 3-year old, lot 1 (A), and last season's seed, lot 59 (B) of the same strain of Cleveland cotton after 24 days at 12° C. Younger seed grown at Clemson College. Test started December 17, 1927

occurs as the seed becomes older, they do suggest that a slight improvement may occur which is distinct from anything in the nature of afterripening.

SUMMARY

The minimum temperature for the germination of cottonseed is approximately 12° C. The increase in activity up to 15° is rapid. Seeds that do not germinate at a given low temperature will often remain viable as long as two months or more under the unfavorable conditions.

Of all the varieties studied Pima and two varieties of *Gossypium nanking* exhibit the most rapid and most complete germination at low temperature. A strain of Cleveland used repeatedly ranks well toward the foot. Most of the upland varieties studied are intermediate, but owing to conflicting evidence it is impossible to rank them among themselves.

There are individual variations in the ability of cottonseed to germinate in the cold, which if hereditary and correlated with seedling hardiness, can be used in selecting for this latter character.

There is some indication that the rate and percentage of germination in the cold increase with the age of the seed, at least for a year or two.

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